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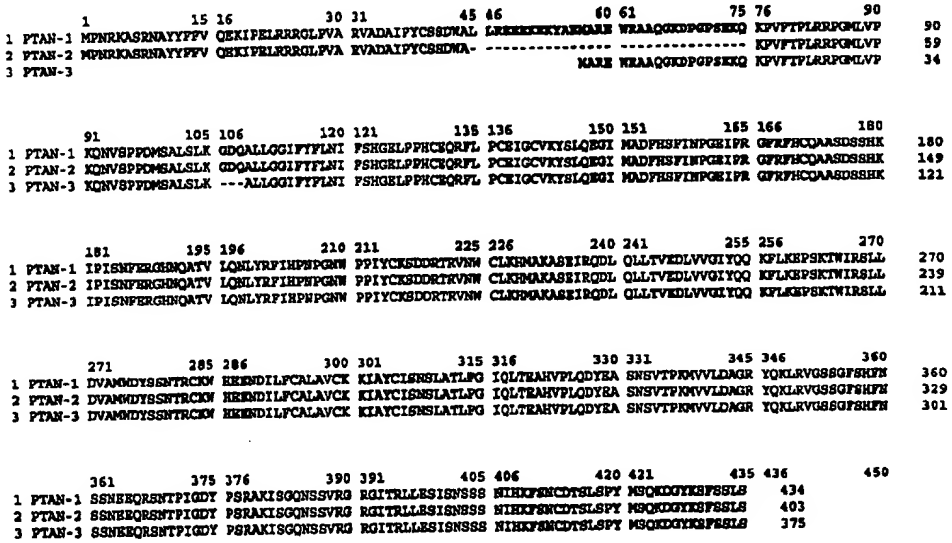

(57) Abstract

Novel testis–specific genes and encoded proteins (PTANs) are described. PTANs are over–expressed in prostate cancer. The nucleotide and amino acid sequences of three distinct PTAN isoforms, designated PTAN–1, PTAN–2 and PTAN–3 are provided. The PTANs show no homology to any known gene. The testis–specific expression profile of PTAN in normal adult tissues, combined with the over–expression observed in prostate tumor xenografts, suggests that PTAN may be aberrantly over–expressed in at least some prostate cancers, and thus may be a useful diagnostic and/or therapeutic target for prostate cancers.

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PTANs: TESTIS SPECIFIC PROTEINS EXPRESSED IN PROSTATE CANCER

FIELD OF THE INVENTION

The invention described herein relates to a novel gene and its encoded proteins, termed PTANs, and to diagnostic and therapeutic methods and compositions useful in the management of various cancers which express PTANs, particularly including prostate and breast cancers.

BACKGROUND OF THE INVENTION

Cancer is the second leading cause of human death next to coronary disease. Worldwide, millions of people die from cancer every year. In the United States alone, cancer causes the death of well over a half-million people annually, with some 1.4 million new cases diagnosed per year. While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death.

Worldwide, several cancers stand out as the leading killers. In particular, carcinomas of the lung, prostate, breast, colon, pancreas, and ovary represent the primary causes of cancer death. These and virtually all other carcinomas share a common lethal feature. With very few exceptions, metastatic disease from a carcinoma is fatal. Moreover, even for those cancer patients who initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the potential for recurrence or treatment failure. Many cancer patients experience physical debilitations following treatment. Many cancer patients experience a recurrence.

Generally speaking, the fundamental problem in the management of the deadliest cancers is the lack of effective and non-toxic systemic therapies. Molecular medicine promises to redefine the ways in which these cancers are managed. Unquestionably, there is an intensive worldwide effort aimed at the development of novel molecular approaches to cancer diagnosis and treatment. For example, there is a great interest in identifying truly tumor-specific genes and proteins that could be used as diagnostic and prognostic markers and/or therapeutic targets or agents. Research efforts in these areas are encouraging, and the increasing availability of useful molecular technologies has accelerated the acquisition of meaningful knowledge about cancer. Nevertheless, progress is slow and generally uneven.

As discussed below, the management of prostate cancer serves as a good example of the limited extent to which molecular biology has translated into real progress in the clinic. With limited exceptions, the situation is more or less the same for the other major carcinomas mentioned above.

Worldwide, prostate cancer is the fourth most prevalent cancer in men. In North America and Northern Europe, it is by far the most common male cancer and is the second leading cause of cancer death in men. In the United States alone, well over 40,000 men die annually of this disease - second only to lung cancer. Despite the magnitude of these figures, there is still no effective treatment for metastatic prostate cancer. Surgical prostatectomy, radiation therapy, hormone ablation therapy, and chemotherapy continue to be the main treatment modalities. Unfortunately, these treatments are ineffective for many and are often associated with undesirable consequences.

On the diagnostic front, the lack of a prostate tumor marker that can accurately detect early-stage, localized tumors remains a significant limitation in the management of this disease. Although the serum PSA assay has been a very useful tool, its specificity and general utility is widely regarded as lacking in several important respects.

Most prostate cancers initially occur in the peripheral zone of the prostate gland, away from the urethra. Tumors within this zone may not produce any symptoms and, as a result, most men with early-stage prostate cancer will not present clinical symptoms of the disease until significant progression has occurred. Tumor progression into the transition zone of the prostate may lead to urethral obstruction, thus producing the first symptoms of the disease. However, these clinical symptoms are indistinguishable from the common non-malignant condition of benign prostatic hyperplasia (BPH). Early detection and diagnosis of prostate cancer currently relies on digital rectal examinations (DRE), prostate specific antigen (PSA) measurements, transrectal ultrasonography (TRUS), and transrectal needle biopsy (TRNB). At present, serum PSA measurement in combination with DRE represent the leading tool used to detect and diagnose prostate cancer. Both have major limitations which have fueled intensive research into finding better diagnostic markers of this disease.

Similarly, there is no available marker that can predict the emergence of the typically fatal metastatic stage of prostate cancer. Diagnosis of metastatic stage is presently achieved by open surgical or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy analysis. Clearly,

better imaging and other less invasive diagnostic methods offer the promise of easing the difficulty those procedures place on a patient, as well as improving diagnostic accuracy and opening therapeutic options. A similar problem is the lack of an effective prognostic marker for determining which cancers are indolent and which ones are or will be aggressive. PSA, for example, fails to discriminate accurately between indolent and aggressive cancers. Until there are prostate tumor markers capable of reliably identifying early-stage disease, predicting susceptibility to metastasis, and precisely imaging tumors, the management of prostate cancer will continue to be extremely difficult.

PSA is the most widely used tumor marker for screening, diagnosis, and monitoring prostate cancer today. In particular, several immunoassays for the detection of serum PSA are in widespread clinical use. Recently, a reverse transcriptase-polymerase chain reaction (RT-PCR) assay for PSA mRNA in serum has been developed. However, PSA is not a disease-specific marker, as elevated levels of PSA are detectable in a large percentage of patients with BPH and prostatitis (25-86%)(Gao et al., 1997, Prostate 31: 264-281), as well as in other nonmalignant disorders and in some normal men, a factor which significantly limits the diagnostic specificity of this marker. For example, elevations in serum PSA of between 4 to 10 ng/ml are observed in BPH, and even higher values are observed in prostatitis, particularly acute prostatitis. BPH is an extremely common condition in men. Further confusing the situation is the fact that serum PSA elevations may be observed without any indication of disease from DRE, and visa-versa. Moreover, it is now recognized that PSA is not prostate-specific (Gao et al., supra, for review).

Various methods designed to improve the specificity of PSA-based detection have been described, such as measuring PSA density and the ratio of free vs. complexed PSA. However, none of these methodologies have been able to reproducibly distinguish benign from malignant prostate disease. In addition, PSA diagnostics have sensitivities of between 57-79% (Cupp & Osterling, 1993, Mayo Clin Proc 68:297-306), and thus miss identifying prostate cancer in a significant population of men with the disease.

There are some known markers which are expressed predominantly in prostate, such as prostate specific membrane antigen (PSM), a hydrolase with 85% identity to a rat neuropeptidase (Carter et al., 1996, Proc. Natl. Acad. Sci. USA 93: 749; Bzdega et al., 1997, J. Neurochem. 69: 2270). However, the expression of PSM in small intestine and brain (Israeli et al., 1994, Cancer Res. 54: 1807), as well its potential role in

neuropeptide catabolism in brain, raises concern of potential neurotoxicity with anti-PSM therapies. Preliminary results using an Indium-111 labeled, anti-PSM monoclonal antibody to image recurrent prostate cancer show some promise (Sodee et al., 1996, Clin Nuc Med 21: 759-766). More recently identified prostate cancer markers include PCTA-1 (Su et al., 1996, Proc. Natl. Acad. Sci. USA 93: 7252) and prostate stem cell antigen (PSCA) (Reiter et al., 1998, Proc. Natl. Acad. Sci. USA 95: 1735). PCTA-1, a novel galectin, is largely secreted into the media of expressing cells and may hold promise as a diagnostic serum marker for prostate cancer (Su et al., 1996). PSCA, a GPI-linked cell surface molecule, was cloned from LAPC-4 cDNA and is unique in that it is expressed primarily in basal cells of normal prostate tissue and in cancer epithelia (Reiter et al., 1998). Vaccines for prostate cancer are also being actively explored with a variety of antigens, including PSM and PSA.

SUMMARY OF THE INVENTION

The present invention relates to a novel, largely testis-specific gene, designated PTAN, which is over-expressed in prostate cancer. RT-PCR, Northern blot and RNA dot blot expression analysis of PTAN gene expression in normal tissues shows a highly testis-specific expression pattern in adult tissues. Analysis of PTAN expression in normal prostate and prostate tumor xenografts shows over-expression in LAPC-4 prostate tumor xenografts. The nucleotide and amino acid sequences of three distinct PTAN isoforms, designated PTAN-1, PTAN-2 and PTAN-3 are shown in FIGS. 1, 2 and 3, respectively. The PTANs show significant homology to several testis-derived ESTs but no homology to any known gene in any public database. The testis-specific expression profile of PTAN in normal adult tissues, combined with the over-expression observed in prostate tumor xenografts, suggests that PTAN may be aberrantly over-expressed in at least some prostate cancers, and thus may be a useful diagnostic and/or therapeutic target for prostate cancers.

The invention provides polynucleotides corresponding or complementary to all or part of the PTAN genes, mRNAs, and/or coding sequences, preferably in isolated form, including polynucleotides encoding PTAN proteins and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to the PTAN genes or mRNA sequences or parts thereof, and polynucleotides or oligonucleotides which hybridize to the PTAN genes, mRNAs, or to PTAN-encoding polynucleotides. Also provided are means for isolating cDNAs and the genes encoding PTAN. Recombinant DNA molecules containing PTAN polynucleotides, cells transformed or transduced with such molecules, and host-vector systems for the expression of PTAN gene products are also provided. The invention further provides

PTAN proteins and polypeptide fragments thereof. The invention further provides antibodies that bind to PTAN proteins and polypeptide fragments thereof, including polyclonal and monoclonal antibodies, murine and other mammalian antibodies, chimeric antibodies, humanized and fully human antibodies, antibodies labeled with a detectable marker. The invention further provides methods for detecting the presence of PTAN polynucleotides and proteins in various biological samples, as well as methods for identifying cells that express PTAN. The invention further provides various therapeutic compositions and strategies for treating cancers which express PTAN such as prostate and breast cancers, including therapies aimed at inhibiting the transcription, translation, processing or function of PTAN and cancer vaccines.

BRIEF DESCRIPTION OF THE FIGURES

FIG. 1. Nucleotide and amino acid sequences of PTAN-1. See Example 2, infra. D2 clone sequence, putative start methionine with Kozak sequence is indicated in bold, the putative nuclear localization signal is boxed and shaded, a 31 a.a. insert with respect to the C6 sequence (FIG. 2) is indicated in bold and underlined.

FIG. 2. Nucleotide and amino acid sequences of PTAN-2. See Example 2, infra. C6 clone sequence, putative start methionine with Kozak sequence is indicated in bold, the putative nuclear localization signal is boxed and shaded.

FIG. 3. Nucleotide and amino acid sequences of PTAN-3. See Example 3, infra. C8 clone sequence, putative start methionine with Kozak sequence is indicated in bold, the putative nuclear localization signal is boxed and shaded.

FIG. 4. Nucleotide and deduced open reading frame amino acid sequences of SSH-isolated PTAN cDNA fragment, clone 26P5C7. Putative start methionine with Kozak sequence is indicated in bold.

FIG. 5. Amino acid sequence alignment of PTAN-1, PTAN-2 and PTAN–3 isoforms using ClustalW1.7. Amino acid differences are indicated in bold type.

FIG. 6. Schematic representation of the three PTAN isoforms.

FIG. 7. Nucleotide (cDNA) sequence alignment of PTAN-1, PTAN-2 and PTAN–3 isoforms using ClustalW1.7.

FIG. 8. RT-PCR analysis of PTAN gene expression in prostate cancer xenografts, normal prostate, and other tissues and cell lines, showing high level expression in LAPC-4 prostate cancer xenografts and lower level expression in normal prostate (Panel A); and showing detectable expression in normal tissues after 30 cycles of PCR amplification is relatively restricted to testis in normal adult tissues (Panels B and C).

FIG. 9. Northern blot analysis of PTAN expression in various normal human tissues showing exclusive expression in testis (using 26P5C7 probe). Size standards in kilobases (kb) are indicated on the side. Each lane contains 2 μg of mRNA. The results show exclusive expression of PTAN in testis.

FIG. 10. An mRNA dot blot analysis of PTAN expression in 76 different samples from human tissues showing exclusive expression in testis (using 26P5C7 probe).

FIG. 11. Northern blot analysis showing PTAN expression in human prostate cancer xenografts and breast cancer cell lines (using 26P5C7 probe). Xenograft and cell line filters were prepared with 10 μg of total RNA per lane. The blots were analyzed using a 26P5C7/PTAN derived gene fragment probe. All RNA samples were normalized by ethidium bromide staining. Kilobases= kb.

FIG. 12. Western blot detection of recombinant human PTAN-1 and PTAN-2 proteins in lysates of 293T cells transfected with a His-tagged PTAN-1 and PTAN-2 cDNAs using anti-PTAN rabbit polyclonal antiserum. Molecular weight standards are indicated on the side in kilodaltons (KD).

FIG. 13. Western blot analysis of subcellular fractions of PTAN expressing 293T cells, showing localization predominantly in the nuclear fraction of cells. Upper Left: Blot probed with 0.1 μg/ml of anti-His Ab. Upper Right: Blot probed with 1 μg/ml of affinity purified anti-PTAN polyclonal Ab. As controls for the enrichment of nuclear proteins in the nuclei fraction, the same lysates were analyzed by Western blot with antibodies specific for the nuclear proteins c-Abl (bottom left) and Cdk2 (bottom right).

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly understood by those of skill in the art to which this invention pertains. In some cases, terms with commonly

understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art. The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized molecular cloning methodologies described in Sambrook et al., Molecular Cloning: A Laboratory Manual 2nd. edition (1989) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. As appropriate, procedures involving the use of commercially available kits and reagents are generally carried out in accordance with manufacturer defined protocols and/or parameters unless otherwise noted.

As used herein, the terms "advanced prostate cancer", "locally advanced prostate cancer", "advanced disease" and "locally advanced disease" mean prostate cancers which have extended through the prostate capsule, and are meant to include stage C disease under the American Urological Association (AUA) system, stage C1 - C2 disease under the Whitmore-Jewett system, and stage T3 - T4 and N+ disease under the TNM (tumor, node, metastasis) system. In general, surgery is not recommended for patients with locally advanced disease, and these patients have substantially less favorable outcomes compared to patients having clinically localized (organ-confined) prostate cancer. Locally advanced disease is clinically identified by palpable evidence of induration beyond the lateral border of the prostate, or asymmetry or induration above the prostate base. Locally advanced prostate cancer is presently diagnosed pathologically following radical prostatectomy if the tumor invades or penetrates the prostatic capsule, extends into the surgical margin, or invades the seminal vesicles.

As used herein, the terms "metastatic prostate cancer" and "metastatic disease" mean prostate cancers which have spread to regional lymph nodes or to distant sites, and are meant to include stage D disease under the AUA system and stage TxNxM+ under the TNM system. As is the case with locally advanced prostate cancer, surgery is generally not indicated for patients with metastatic disease, and hormonal (androgen ablation) therapy is the preferred treatment modality. Patients with metastatic prostate cancer eventually develop an androgen-refractory state within 12 to 18 months of treatment initiation, and approximately half of these patients die within 6 months thereafter. The most common site for prostate cancer metastasis is bone. Prostate cancer bone metastases are, on balance, characteristically osteoblastic rather than osteolytic (i.e., resulting in net bone formation). Bone metastases are found most frequently in the spine, followed by the femur, pelvis, rib cage, skull and

humerus. Other common sites for metastasis include lymph nodes, lung, liver and brain. Metastatic prostate cancer is typically diagnosed by open or laparoscopic pelvic lymphadenectomy, whole body radionuclide scans, skeletal radiography, and/or bone lesion biopsy.

As used herein, the term "polynucleotide" means a polymeric form of nucleotides of at least 10 bases or base pairs in length, either ribonucleotides or deoxynucleotides or a modified form of either type of nucleotide, and is meant to include single and double stranded forms of DNA.

As used herein, the term "polypeptide" means a polymer of at least 10 amino acids. Throughout the specification, standard three letter or single letter designations for amino acids are used.

As used herein, the terms "hybridize", "hybridizing", "hybridizes" and the like, used in the context of polynucleotides, are meant to refer to conventional hybridization conditions, preferably such as hybridization in 50% formamide/6XSSC/0.1% SDS/100 µg/ml ssDNA, in which temperatures for hybridization are above 37 degrees C and temperatures for washing in 0.1XSSC/0.1% SDS are above 55 degrees C, and most preferably to stringent hybridization conditions.

In the context of amino acid sequence comparisons, the term "identity" is used to express the percentage of amino acid residues at the same relative position which are the same. Also in this context, the term "homology" is used to express the percentage of amino acid residues at the same relative positions which are either identical or are similar, using the conserved amino acid criteria of BLAST analysis, as is generally understood in the art. Further details regarding amino acid substitutions, which are considered conservative under such criteria, are provided below.

Additional definitions are provided throughout the subsections which follow.

STRUCTURE AND EXPRESSION OF PTANs

As is further described in the Examples which follow, the PTAN genes and proteins have been characterized using a number of analytical approaches. For example, analyses of nucleotide coding and amino acid sequences were conducted in order to identify potentially related molecules and three distinct PTAN isoforms, as well as recognizable structural domains, topological features, and other elements within the

PTAN mRNA and protein structures. RT-PCR, Northern blot and RNA dot blot analyses of PTAN mRNA expression were conducted in order to establish the range of normal and cancerous tissues expressing PTAN message. Western blot analyses were used to characterize anti-PTAN antibody preparations.

A comparative schematic diagram of the three PTAN protein isoform structures is shown in FIG. 6. The nucleotide and amino acid sequences of the full length cDNA clones corresponding to PTAN-1 (1690 bp), PTAN-2 (1640 bp) and PTAN-3 (1760 bp) are shown in FIGS. 1, 2 and 3, respectively. These cDNAs encode open reading frames (ORF) of 434, 403 and 375 amino acids, respectively. Sequence analysis shows no significant homology to any known genes. PTAN-1 and PTAN-2 have a nuclear localization signal (NLS) at the amino-terminus (FIG. 1, 2). PTAN-1 differs from PTAN-2 by containing a 31 a.a. domain (residues 45-75) that is absent in PTAN-2 (FIG. 1, FIG. 5). PTAN-3 lacks the amino-terminal NLS, exhibits its start methionine within the 31 a.a. domain unique to PTAN-1 and exhibits a 3 amino acid deletion with respect to PTAN-1 and PTAN-2 (residues 106-108 in PTAN-1) (FIG. 5).

The differences between the PTAN isoforms are shown in an alignment in FIG. 5. Differences between the PTAN isoforms are also detected in the 5' untranslated regions (UTRs). The 5'UTRs for PTAN-1 and PTAN-2 are the same, while the 5'UTR for PTAN-3 is significantly different (FIG 7). In all three PTANs, the 5'UTRs contain sequences rich in GC content (68-70%) indicating that these sequences contain regulatory elements. The PTAN proteins may be factors that potentially regulate transcription.

Recombinant PTAN-1 is expressed as a 49 kD protein in a mammalian expression system. Recombinant PTAN-2 is expressed as a 46 kD protein in the same system. The human PTAN gene maps to chromosome 1q22.

PTAN expression is testis-specific in normal adult human tissues (FIGS. 9 and 10), but is also expressed in certain cancers, including prostate and breast cancers. (FIG. 11). Human prostate tumor xenografts originally derived from a patient with high grade metastatic prostate cancer express high levels of PTAN (FIG. 11). Lower level PTAN expression is detected in breast cancer cell lines, suggesting that PTAN is a highly testis-specific gene that may be up-regulated in various human cancers.

PTAN POLYNUCLEOTIDES

One aspect of the invention provides polynucleotides corresponding or complementary to all or part of a PTAN gene, mRNA, and/or coding sequence, preferably in isolated form, including polynucleotides encoding a PTAN protein and fragments thereof, DNA, RNA, DNA/RNA hybrid, and related molecules, polynucleotides or oligonucleotides complementary to a PTAN gene or mRNA sequence or a part thereof, and polynucleotides or oligonucleotides which hybridize to a PTAN gene, mRNA, or to a PTAN -encoding polynucleotide (collectively, "PTAN polynucleotides"). As used herein, the PTAN gene and protein is meant to include the PTAN genes and proteins specifically described herein and the genes and proteins corresponding to other PTAN proteins and structurally similar variants of the foregoing. Such other PTAN proteins and variants will generally have coding sequences which are highly homologous to the PTAN coding sequence, and preferably will share at least about 50% amino acid identity and at least about 60% amino acid homology (using BLAST criteria), more preferably sharing 70% or greater homology (using BLAST criteria).

One embodiment of a PTAN polynucelotide is a PTAN-1 polynucleotide having the sequence shown in FIG. 1. Another embodiment is a PTAN-2 polynucleotide having the nucleotide sequence shown in FIG. 2. Another embodiment is a PTAN-2 polynucleotide having the nucleotide sequence shown in FIG. 3.

A PTAN polynucleotide may comprise a polynucleotide having the nucleotide sequence of human PTAN-1 as shown in FIG. 1, wherein T can also be U; a polynucleotide which encodes all or part of the PTAN-1 protein; a sequence complementary to the foregoing; or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide having the sequence as shown in FIG. 1, from nucleotide residue number 26 through nucleotide residue number 1327, wherein T can also be U. Another embodiment comprises a polynucleotide encoding a PTAN-1 polypeptide whose sequence is encoded by the cDNA contained in the plasmid as deposited with American Type Culture Collection as Accession No. 98976. Another embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the human PTAN-1 cDNA shown in FIG. 1 or to a polynucleotide fragment thereof.

Similarly, a PTAN polynucleotide may comprise a polynucleotide having the nucleotide sequence of human PTAN-2 as shown in FIG. 2, wherein T can also be U; a polynucleotide which encodes all or part of the PTAN-2 protein; a sequence

complementary to the foregoing; or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide having the sequence as shown in FIG. 2, from nucleotide residue number 61 through nucleotide residue number 1269, wherein T can also be U. Another embodiment comprises a polynucleotide encoding a PTAN-2 polypeptide whose sequence is encoded by the cDNA contained in the plasmid as deposited with American Type Culture Collection as Accession No. 98977. Another embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the human PTAN-2 cDNA shown in FIG. 2 or to a polynucleotide fragment thereof.

Further, a PTAN polynucleotide may comprise a polynucleotide having the nucleotide sequence of human PTAN-3 as shown in FIG. 3, wherein T can also be U; a polynucleotide which encodes all or part of the PTAN-3 protein; a sequence complementary to the foregoing; or a polynucleotide fragment of any of the foregoing. Another embodiment comprises a polynucleotide having the sequence as shown in FIG. 3, from nucleotide residue number 271 through nucleotide residue number 1395, wherein T can also be U. Another embodiment comprises a polynucleotide encoding a PTAN-3 polypeptide whose sequence is encoded by the cDNA contained in the plasmid as deposited with American Type Culture Collection as Accession No. 207095. Another embodiment comprises a polynucleotide which is capable of hybridizing under stringent hybridization conditions to the human PTAN-3 cDNA shown in FIG. 3 or to a polynucleotide fragment thereof.

Specifically contemplated are genomic DNA, cDNAs, ribozymes, and antisense molecules, as well as nucleic acid molecules based on an alternative backbone or including alternative bases, whether derived from natural sources or synthesized. For example, antisense molecules can be RNAs or other molecules, including peptide nucleic acids (PNAs) or non-nucleic acid molecules such as phosphorothioate derivatives, that specifically bind DNA or RNA in a base pair-dependent manner. A skilled artisan can readily obtain these classes of nucleic acid molecules using the PTAN polynucleotides and polynucleotide sequences disclosed herein.

Further specific embodiments of this aspect of the invention include primers and primer pairs, which allow the specific amplification of the polynucleotides of the invention or of any specific parts thereof, and probes that selectively or specifically hybridize to nucleic acid molecules of the invention or to any part thereof. Probes may be labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator

or enzyme. Such probes and primers can be used to detect the presence of a PTAN polynucleotide in a sample and as a means for detecting a cell expressing a PTAN protein. Examples of such probes include polypeptides comprising all or part of the human PTAN cDNA sequences shown in FIGS. 1, 2 and 3. Examples of primer pairs capable of specifically amplifying PTAN mRNAs are also described in the Examples which follow. As will be understood by the skilled artisan, a great many different primers and probes may be prepared based on the sequences provided in herein and used effectively to amplify and/or detect a PTAN mRNA.

As used herein, a polynucleotide is said to be "isolated" when it is substantially separated from contaminant polynucleotides which correspond or are complementary to genes other than the PTAN gene or which encode polypeptides other than PTAN gene product or fragments thereof. A skilled artisan can readily employ nucleic acid isolation procedures to obtain an isolated PTAN polynucleotide.

The PTAN polynucleotides of the invention are useful for a variety of purposes, including but not limited to their use as probes and primers for the amplification and/or detection of the PTAN gene(s), mRNA(s), or fragments thereof; as reagents for the diagnosis and/or prognosis of prostate cancer and other cancers; as coding sequences capable of directing the expression of PTAN polypeptides; as tools for modulating or inhibiting the expression of the PTAN gene(s) and/or translation of the PTAN transcript(s); and as therapeutic agents.

METHODS FOR ISOLATING PTAN-ENCODING NUCLEIC ACID MOLECULES

The PTAN cDNA sequences described herein enable the isolation of other polynucleotides encoding PTAN gene product(s), as well as the isolation of polynucleotides encoding PTAN gene product homologues, alternatively spliced isoforms, allelic variants, and mutant forms of the PTAN gene product. Various molecular cloning methods that can be employed to isolate full length cDNAs encoding a PTAN gene are well known (See, for example, Sambrook, J. et al., Molecular Cloning: A Laboratory Manual, 2d edition., Cold Spring Harbor Press, New York, 1989; Current Protocols in Molecular Biology. Ausubel et al., Eds., Wiley and Sons, 1995). For example, lambda phage cloning methodologies may be conveniently employed, using commercially available cloning systems (e.g., Lambda ZAP Express, Stratagene). Phage clones containing PTAN gene cDNAs may be identified by probing with a labeled PTAN cDNA or a fragment thereof. For example, in one embodiment, the PTAN cDNA (FIG. 1, 2, 3) or a portion thereof can be synthesized and used as a probe to retrieve overlapping and full length cDNAs corresponding to a PTAN gene. The PTAN gene itself may be

isolated by screening genomic DNA libraries, bacterial artificial chromosome libraries (BACs), yeast artificial chromosome libraries (YACs), and the like, with PTAN DNA probes or primers.

RECOMBINANT DNA MOLECULES AND HOST-VECTOR SYSTEMS

The invention also provides recombinant DNA or RNA molecules containing a PTAN polynucleotide, including but not limited to phages, plasmids, phagemids, cosmids, YACs, BACs, as well as various viral and non-viral vectors well known in the art, and cells transformed or transfected with such recombinant DNA or RNA molecules. As used herein, a recombinant DNA or RNA molecule is a DNA or RNA molecule that has been subjected to molecular manipulation in vitro. Methods for generating such molecules are well known (see, for example, Sambrook et al, 1989, supra).

The invention further provides a host-vector system comprising a recombinant DNA molecule containing a PTAN polynucleotide within a suitable prokaryotic or eukaryotic host cell. Examples of suitable eukaryotic host cells include a yeast cell, a plant cell, or an animal cell, such as a mammalian cell or an insect cell (e.g., a baculovirus-infectible cell such as an Sf9 or HighFive cell). Examples of suitable mammalian cells include various prostate cancer cell lines such LnCaP, PC-3, DU145, LAPC-4, TsuPr1, other transfectable or transducible prostate cancer cell lines, as well as a number of mammalian cells routinely used for the expression of recombinant proteins (e.g., COS, CHO, 293, 293T cells). More particularly, a polynucleotide comprising the coding sequence of a PTAN may be used to generate PTAN proteins or fragments thereof using any number of host-vector systems routinely used and widely known in the art.

A wide range of host-vector systems suitable for the expression of PTAN proteins or fragments thereof are available, see for example, Sambrook et al., 1989, supra; Current Protocols in Molecular Biology, 1995, supra). Preferred vectors for mammalian expression include but are not limited to pcDNA 3.1 myc-His-tag (Invitrogen) and the retroviral vector pSRαtkneo (Muller et al., 1991, MCB 11:1785). Using these expression vectors, PTAN may be preferably expressed in several prostate cancer and non-prostate cell lines, including for example 293, 293T, rat-1, 3T3, PC-3, LNCaP and TsuPr1. The host-vector systems of the invention are useful for the production of a PTAN protein or fragment thereof. Such host-vector systems may be employed to study the functional properties of PTAN and PTAN mutations.

Recombinant human PTAN protein may be produced by mammalian cells transfected with a construct encoding PTAN. In a particular embodiment described in the Examples, 293T cells are transfected with an expression plasmid encoding PTAN, the PTAN protein is expressed in the 293T cells, and the recombinant PTAN protein is isolated using standard purification methods (e.g., affinity purification using anti-PTAN antibodies). In another embodiment, also described in the Examples herein, the PTAN coding sequence is subcloned into the retroviral vector pSRαMSVtkneo and used to infect various mammalian cell lines, including 3T3CL7, PC3 and LnCaP in order to establish PTAN expressing cell lines. Various other expression systems well known in the art may also be employed. Expression constructs encoding a leader peptide joined in frame to the PTAN coding sequence may be used for the generation of a secreted form of recombinant PTAN protein.

Proteins encoded by the PTAN genes, or by fragments thereof, will have a variety of uses, including but not limited to generating antibodies and in methods for identifying ligands and other agents (i.e., other bHLH proteins) and cellular constituents that bind to a PTAN gene product. Antibodies raised against a PTAN protein or fragment thereof may be useful in diagnostic and prognostic assays, and imaging methodologies in the management of human cancers characterized by expression of PTAN protein, including but not limited to cancers of the prostate and breast. Such antibodies may be expressed intracellularly and used in methods of treating patients with such cancers. Various immunological assays useful for the detection of PTAN proteins are contemplated, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), immunocytochemical methods, and the like. Such antibodies may be labeled and used as immunological imaging reagents capable of detecting PTAN expressing cells (e.g., in radioscintigraphic imaging methods). PTAN proteins may also be particularly useful in generating cancer vaccines, as further described below.

PTAN PROTEINS

Another aspect of the present invention provides PTAN proteins and polypeptide fragments thereof. The PTAN proteins of the invention include those specifically identified herein, as well as allelic variants, conservative substitution variants and homologs that can be isolated/generated and characterized without undue experimentation following the methods outlined below. Fusion proteins which combine parts of different PTAN proteins or fragments thereof, as well as fusion proteins of a PTAN protein and a heterologous polypeptide are also included. Such PTAN proteins

will be collectively referred to as the PTAN proteins, the proteins of the invention, or PTAN. As used herein, the term "PTAN polypeptide" refers to a polypeptide fragment or a PTAN protein of at least 10 amino acids, preferably at least 15 amino acids.

Specific embodiments of PTAN proteins comprises a polypeptide having the amino acid sequence of human PTAN1, -2, and -3 as shown in FIGS. 1, 2 and 3, respectively.

In general, naturally occurring allelic variants of human PTAN will share a high degree of structural identity and homology (e.g., 90% or more identity). Typically, allelic variants of the PTAN proteins will contain conservative amino acid substitutions within the PTAN sequences described herein or will contain a substitution of an amino acid from a corresponding position in a PTAN homologue. One class of PTAN allelic variants will be proteins that share a high degree of homology with at least a small region of a particular PTAN amino acid sequence, but will further contain a radical departure form the sequence, such as a non-conservative substitution, truncation, insertion or frame shift.

Conservative amino acid substitutions can frequently be made in a protein without altering either the conformation or the function of the protein. Such changes include substituting any of isoleucine (I), valine (V), and leucine (L) for any other of these hydrophobic amino acids; aspartic acid (D) for glutamic acid (E) and vice versa; glutamine (Q) for asparagine (N) and vice versa; and serine (S) for threonine (T) and vice versa. Other substitutions can also be considered conservative, depending on the environment of the particular amino acid and its role in the three-dimensional structure of the protein. For example, glycine (G) and alanine (A) can frequently be interchangeable, as can alanine (A) and valine (V). Methionine (M), which is relatively hydrophobic, can frequently be interchanged with leucine and isoleucine, and sometimes with valine. Lysine (K) and arginine (R) are frequently interchangeable in locations in which the significant feature of the amino acid residue is its charge and the differing pK's of these two amino acid residues are not significant. Still other changes can be considered "conservative" in particular environments.

PTAN proteins may be embodied in many forms, preferably in isolated form. As used herein, a protein is said to be "isolated" when physical, mechanical or chemical methods are employed to remove the PTAN protein from cellular constituents that are normally associated with the protein. A skilled artisan can readily employ standard purification methods to obtain an isolated PTAN protein. A purified PTAN protein molecule will be substantially free of other proteins or molecules which impair the binding of PTAN to antibody or other ligand. The nature and degree of isolation and purification will depend

on the intended use. Embodiments of a PTAN protein include a purified PTAN protein and a functional, soluble PTAN protein. In one form, such functional, soluble PTAN proteins or fragments thereof retain the ability to bind antibody or other ligand.

The invention also provides PTAN polypeptides comprising biologically active fragments of the PTAN amino acid sequence, such as a polypeptide corresponding to part of the amino acid sequences for PTAN as shown in FIGS. 1, 2 and 3. Such polypeptides of the invention exhibit properties of the PTAN protein, such as the ability to elicit the generation of antibodies which specifically bind an epitope associated with the PTAN protein.

PTAN polypeptides can be generated using standard peptide synthesis technology or using chemical cleavage methods well known in the art based on the amino acid sequences of the human PTAN proteins disclosed herein. Alternatively, recombinant methods can be used to generate nucleic acid molecules that encode a polypeptide fragment of a PTAN protein. In this regard, the PTAN-encoding nucleic acid molecules described herein provide means for generating defined fragments of PTAN proteins. PTAN polypeptides are particularly useful in generating and characterizing domain specific antibodies (e.g., antibodies recognizing an extracellular or intracellular epitope of a PTAN protein), in identifying agents or cellular factors that bind to PTAN or a particular structural domain thereof, and in various therapeutic contexts, including but not limited to cancer vaccines.

Polypeptides comprising amino acid sequences which are unique to a particular PTAN protein (relative to other PTAN proteins) may be used to generate antibodies which will specifically react with that particular PTAN protein. For example, referring to the amino acid alignment of the PTANs shown in FIG. 5, the skilled artisan will readily appreciate that each molecule contains stretches of sequence unique to its structure. These unique stretches can be used to generate PTAN-1, PTAN-2 or PTAN-3 specific antibodies.

PTAN polypeptides containing particularly interesting structures can be predicted and/or identified using various analytical techniques well known in the art, including, for example, the methods of Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis, or on the basis of immunogenicity. Fragments containing such structures are particularly useful in generating subunit specific anti-PTAN antibodies or in identifying cellular factors that bind to PTAN.

In a specific embodiment described in the examples which follow, PTAN is conveniently expressed in 293T cells transfected with a CMV-driven expression vector encoding PTAN with a C-terminal 6XHis and MYC tag (pcDNA3.1/mycHIS, Invitrogen). The secreted HIS-tagged PTAN in the culture media may be purified using a nickel column using standard techniques.

PTAN ANTIBODIES

Another aspect of the invention provides antibodies that bind to PTAN proteins and polypeptides. The most preferred antibodies will specifically bind to a PTAN protein and will not bind (or will bind weakly) to non-PTAN proteins and polypeptides. Anti-PTAN antibodies that are particularly contemplated include monoclonal and polyclonal antibodies as well as fragments containing the antigen binding domain and/or one or more complementarity determining regions of these antibodies. As used herein, an antibody fragment is defined as at least a portion of the variable region of the immunoglobulin molecule which binds to its target, i.e., the antigen binding region.

PTAN antibodies of the invention may be particularly useful in prostate cancer diagnostic and prognostic assays, and imaging methodologies. Intracellularly expressed antibodies (e.g., single chain antibodies) may be therapeutically useful in treating cancers in which the expression of PTAN is involved, such as for example advanced and metastatic prostate cancers. Similarly, such antibodies may be useful in the treatment, diagnosis, and/or prognosis of other cancers, to the extent PTAN is also expressed or overexpressed in other types of cancer. Other cancers that expresses PTAN include without limitation breast cancer.

The invention also provides various immunological assays useful for the detection and quantification of PTAN and mutant PTAN proteins and polypeptides. Such assays generally comprise one or more PTAN antibodies capable of recognizing and binding a PTAN or mutant PTAN protein, as appropriate, and may be performed within various immunological assay formats well known in the art, including but not limited to various types of radioimmunoassays, enzyme-linked immunosorbent assays (ELISA), enzyme-linked immunofluorescent assays (ELIFA), and the like. In addition, immunological imaging methods capable of detecting prostate cancer and other cancers expressing PTAN (e.g., breast cancer) are also provided by the invention, including but limited to radioscintigraphic imaging methods using labeled PTAN antibodies. Such assays may be clinically useful in the detection, monitoring, and prognosis of PTAN expressing cancers such as prostate cancer.

PTAN antibodies may also be used in methods for purifying PTAN and mutant PTAN proteins and polypeptides and for isolating PTAN homologues and related molecules. For example, in one embodiment, the method of purifying a PTAN protein comprises incubating a PTAN antibody, which has been coupled to a solid matrix, with a lysate or other solution containing PTAN under conditions which permit the PTAN antibody to bind to PTAN; washing the solid matrix to eliminate impurities; and eluting the PTAN from the coupled antibody. Other uses of the PTAN antibodies of the invention include generating anti-idiotypic antibodies that mimic the PTAN protein.

Various methods for the preparation of antibodies are well known in the art. For example, antibodies may be prepared by immunizing a suitable mammalian host using a PTAN protein, peptide, or fragment, in isolated or immunoconjugated form (Antibodies: A Laboratory Manual, CSH Press, Eds., Harlow, and Lane (1988); Harlow, Antibodies, Cold Spring Harbor Press, NY (1989)). In addition, fusion proteins of PTAN may also be used, such as a PTAN GST-fusion protein. In a particular embodiment, a GST fusion protein comprising all or most of the open reading frame amino acid sequence of FIG. 2 may be produced and used as an immunogen to generate appropriate antibodies. In another embodiment, a PTAN peptide may be synthesized and used as an immunogen. As described in Example 5, below, the 15-mer PTAN peptide HSSKEKLRRERIKYC was conjugated to keyhole limpet hemocyanin (KLH) and used to immunize a rabbit. The resulting polyclonal antiserum specifically recognized PTAN expressed in a recombinant mammalian expression system.

In addition, naked DNA immunization techniques known in the art may be used (with or without purified PTAN protein or PTAN expressing cells) to generate an immune response to the encoded immunogen (for review, see Donnelly et al., 1997, Ann. Rev. Immunol. 15: 617-648).

The amino acid sequence of the PTANs as shown in FIGS. 1-3 may be used to select specific regions of the PTAN protein for generating antibodies. For example, hydrophobicity and hydrophilicity analyses of the PTAN amino acid sequence may be used to identify hydrophilic regions in the PTAN structure. Regions of the PTAN protein that show immunogenic structure, as well as other regions and domains, can readily be identified using various other methods known in the art, such as Chou-Fasman, Garnier-Robson, Kyte-Doolittle, Eisenberg, Karplus-Schultz or Jameson-Wolf analysis. Methods for the generation of PTAN antibodies are further illustrated by way of the examples provided herein.

Methods for preparing a protein or polypeptide for use as an immunogen and for preparing immunogenic conjugates of a protein with a carrier such as BSA, KLH, or other carrier proteins are well known in the art. In some circumstances, direct conjugation using, for example, carbodiimide reagents may be used; in other instances linking reagents such as those supplied by Pierce Chemical Co., Rockford, IL, may be effective. Administration of a PTAN immunogen is conducted generally by injection over a suitable time period and with use of a suitable adjuvant, as is generally understood in the art. During the immunization schedule, titers of antibodies can be taken to determine adequacy of antibody formation.

PTAN monoclonal antibodies are preferred and may be produced by various means well known in the art. For example, immortalized cell lines which secrete a desired monoclonal antibody may be prepared using the standard hybridoma technology of Kohler and Milstein or modifications which immortalize producing B cells, as is generally known. The immortalized cell lines secreting the desired antibodies are screened by immunoassay in which the antigen is the PTAN protein or a PTAN fragment. When the appropriate immortalized cell culture secreting the desired antibody is identified, the cells may be expanded and antibodies produced either from in vitro cultures or from ascites fluid.

The antibodies or fragments may also be produced, using current technology, by recombinant means. Regions that bind specifically to the desired regions of the PTAN protein can also be produced in the context of chimeric or CDR grafted antibodies of multiple species origin. Humanized or human PTAN antibodies may also be produced and are preferred for use in therapeutic contexts. Methods for humanizing murine and other non-human antibodies by substituting one or more of the non-human antibody CDRs for corresponding human antibody sequences are well known (see for example, Jones et al., 1986, Nature 321: 522-525; Riechmnan et al., 1988, Nature 332: 323-327; Verhoeyen et al., 1988, Science 239: 1534-1536). See also, Carter et al., 1993, Proc. Natl. Acad. Sci. USA 89: 4285 and Sims et al., 1993, J. Immunol. 151: 2296. Methods for producing fully human monoclonal antibodies include phage display and transgenic methods (for review, see Vaughan et al., 1998, Nature Biotechnology 16: 535-539).

Fully human PTAN monoclonal antibodies may be generated using cloning technologies employing large human Ig gene combinatorial libraries (i.e., phage display) (Griffiths and Hoogenboom, Building an in vitro immune system: human antibodies from phage display libraries. In: Protein Engineering of Antibody Molecules for Prophylactic and Therapeutic Applications in Man. Clark, M. (Ed.), Nottingham Academic, pp 45-64

(1993); Burton and Barbas, Human Antibodies from combinatorial libraries. Id., pp 65-82). Fully human PTAN monoclonal antibodies may also be produced using transgenic mice engineered to contain human immunoglobulin gene loci as described in PCT Patent Application WO98/24893, Kucherlapati and Jakobovits et al., published December 3, 1997 (see also, Jakobovits, 1998, Exp. Opin. Invest. Drugs 7(4): 607-614). This method avoids the in vitro manipulation required with phage display technology and efficiently produces high affinity authentic human antibodies.

Reactivity of PTAN antibodies with a PTAN protein may be established by a number of well known means, including Western blot, immunoprecipitation, ELISA, and FACS analyses using, as appropriate, PTAN proteins, peptides, PTAN-expressing cells or extracts thereof.

A PTAN antibody or fragment thereof of the invention may be labeled with a detectable marker or conjugated to a second molecule. Suitable detectable markers include, but are not limited to, a radioisotope, a fluorescent compound, a bioluminescent compound, chemiluminescent compound, a metal chelator or an enzyme. Further, bi-specific antibodies specific for two or more PTAN epitopes may be generated using methods generally known in the art. Homodimeric antibodies may also be generated by cross-linking techniques known in the art (e.g., Wolff et al., Cancer Res. 53: 2560-2565).

METHODS FOR THE DETECTION OF PTAN

Another aspect of the present invention relates to methods for detecting PTAN polynucleotides and PTAN proteins, as well as methods for identifying a cell which expresses PTAN.

More particularly, the invention provides assays for the detection of PTAN polynucleotides in a biological sample, such as serum, bone, prostate, and other tissues, urine, semen, cell preparations, and the like. Detectable PTAN polynucleotides include, for example, a PTAN gene or fragments thereof, PTAN mRNA, alternative splice variant PTAN mRNAs, and recombinant DNA or RNA molecules containing a PTAN polynucleotide. A number of methods for amplifying and/or detecting the presence of PTAN polynucleotides are well known in the art and may be employed in the practice of this aspect of the invention.

In one embodiment, a method for detecting a PTAN mRNA in a biological sample comprises producing cDNA from the sample by reverse transcription using at least

one primer; amplifying the cDNA so produced using a PTAN polynucleotides as sense and antisense primers to amplify PTAN cDNAs therein; and detecting the presence of the amplified PTAN cDNA. In another embodiment, a method of detecting a PTAN gene in a biological sample comprises first isolating genomic DNA from the sample; amplifying the isolated genomic DNA using PTAN polynucleotides as sense and antisense primers to amplify the PTAN gene therein; and detecting the presence of the amplified PTAN gene. Any number of appropriate sense and antisense probe combinations may be designed from the nucleotide sequences provided for the PTANs (FIGS. 1-3) and used for this purpose.

The invention also provides assays for detecting the presence of a PTAN protein in a tissue of other biological sample such as serum, bone, prostate, and other tissues, urine, cell preparations, and the like. Methods for detecting a PTAN protein are also well known and include, for example, immunoprecipitation, immunohistochemical analysis, Western Blot analysis, molecular binding assays, ELISA, ELIFA and the like. For example, in one embodiment, a method of detecting the presence of a PTAN protein in a biological sample comprises first contacting the sample with a PTAN antibody, a PTAN-reactive fragment thereof, or a recombinant protein containing an antigen binding region of a PTAN antibody; and then detecting the binding of PTAN protein in the sample thereto.

Methods for identifying a cell which expresses PTAN are also provided. In one embodiment, an assay for identifying a cell which expresses a PTAN gene comprises detecting the presence of PTAN mRNA in the cell. Methods for the detection of particular mRNAs in cells are well known and include, for example, hybridization assays using complementary DNA probes (such as in situ hybridization using labeled PTAN riboprobes, Northern blot and related techniques) and various nucleic acid amplification assays (such as RT-PCR using complementary primers specific for PTAN, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like). Alternatively, an assay for identifying a cell which expresses a PTAN gene comprises detecting the presence of PTAN protein in the cell or secreted by the cell. Various methods for the detection of proteins are well known in the art and may be employed for the detection of PTAN proteins and PTAN expressing cells.

PTAN expression analysis may also be useful as a tool for identifying and evaluating agents which modulate PTAN gene expression. For example, PTAN expression is significantly upregulated in prostate cancer, and may also be expressed in other cancers. Identification of a molecule or biological agent that could inhibit PTAN

expression or over-expression in cancer cells may be of therapeutic value. Such an agent may be identified by using a screen that quantifies PTAN expression by RT-PCR, nucleic acid hybridization or antibody binding.

ASSAYS FOR DETERMINING PTAN EXPRESSION STATUS

Determining the status of PTAN expression patterns in an individual may be used to diagnose cancer and may provide prognostic information useful in defining appropriate therapeutic options. Similarly, the expression status of PTAN may provide information useful for predicting susceptibility to particular disease stages, progression, and/or tumor aggressiveness. The invention provides methods and assays for determining PTAN expression status and diagnosing cancers which express PTAN, such as cancers of the prostate and breast. PTAN expression status in patient samples may be analyzed by a number of means well known in the art, including without limitation, immunohistochemical analysis, in situ hybridization, RT-PCR analysis on laser capture micro-dissected samples, western blot analysis of clinical samples and cell lines, and tissue array analysis.

In one aspect, the invention provides assays useful in determining the presence of cancer in an individual, comprising detecting a significant increase in PTAN mRNA or protein expression in a test cell or tissue sample relative to expression levels in the corresponding normal cell or tissue. The presence of PTAN mRNA may, for example, be evaluated in tissue samples including but not limited to colon, lung, prostate, pancreas, bladder, breast, ovary, cervix, testis, head and neck, brain, stomach, etc. The presence of significant PTAN expression in any of these tissues may be useful to indicate the emergence, presence and/or severity of these cancers, since the corresponding normal tissues do not express PTAN mRNA or express it at lower levels.

In a related embodiment, PTAN expression status may be determined at the protein level rather than at the nucleic acid level. For example, such a method or assay would comprise determining the level of PTAN protein expressed by cells in a test tissue sample and comparing the level so determined to the level of PTAN expressed in a corresponding normal sample. In one embodiment, the presence of PTAN protein is evaluated, for example, using immunohistochemical methods. PTAN antibodies or binding partners capable of detecting PTAN protein expression may be used in a variety of assay formats well known in the art for this purpose.

In addition, peripheral blood may be conveniently assayed for the presence of cancer cells, including but not limited to prostate and breast cancers, using RT-PCR to detect PTAN expression. The presence of RT-PCR amplifiable PTAN mRNA provides an indication of the presence of the cancer. RT-PCR detection assays for tumor cells in peripheral blood are currently being evaluated for use in the diagnosis and management of a number of human solid tumors. In the prostate cancer field, these include RT-PCR assays for the detection of cells expressing PSA and PSM (Verkaik et al., 1997, Urol. Res. 25: 373-384; Ghossein et al., 1995, J. Clin. Oncol. 13: 1195-2000; Heston et al., 1995, Clin. Chem. 41: 1687-1688). RT-PCR assays are well known in the art.

A related aspect of the invention is directed to predicting susceptibility to developing cancer in an individual. In one embodiment, a method for predicting susceptibility to cancer comprises detecting PTAN mRNA or PTAN protein in a tissue sample, its presence indicating susceptibility to cancer, wherein the degree of PTAN mRNA expression present is proportional to the degree of susceptibility. In a specific embodiment, the presence of PTAN in prostate tissue is examined, with the presence of PTAN in the sample providing an indication of prostate cancer susceptibility (or the emergence or existence of a prostate tumor). In another specific embodiment, the presence of PTAN in breast tissue is examined, with the presence of PTAN in the sample providing an indication of breast cancer susceptibility (or the emergence or existence of a breast tumor).

Yet another related aspect of the invention is directed to methods for gauging tumor aggressiveness. In one embodiment, a method for gauging aggressiveness of a tumor comprises determining the level of PTAN mRNA or PTAN protein expressed by cells in a sample of the tumor, comparing the level so determined to the level of PTAN mRNA or PTAN protein expressed in a corresponding normal tissue taken from the same individual or a normal tissue reference sample, wherein the degree of PTAN mRNA or PTAN protein expression in the tumor sample relative to the normal sample indicates the degree of aggressiveness. In a specific embodiment, aggressiveness of prostate or breast tumors is evaluated by determining the extent to which PTAN is expressed in the tumor cells, with higher expression levels indicating more aggressive tumors.

Methods for detecting and quantifying the expression of PTAN mRNA or protein are described herein and use standard nucleic acid and protein detection and quantification technologies well known in the art. Standard methods for the detection and quantification of PTAN mRNA include in situ hybridization using labeled PTAN riboprobes, Northern blot and related techniques using PTAN polynucleotide probes,

RT-PCR analysis using primers specific for PTAN, and other amplification type detection methods, such as, for example, branched DNA, SISBA, TMA and the like. In a specific embodiment, semi-quantitative RT-PCR may be used to detect and quantify PTAN mRNA expression as described in the Examples which follow. Any number of primers capable of amplifying PTAN may be used for this purpose, including but not limited to the various primer sets specifically described herein. Standard methods for the detection and quantification of protein may be used for this purpose. In a specific embodiment, polyclonal or monoclonal antibodies specifically reactive with the wild-type PTAN protein may be used in an immunohistochemical assay of biopsied tissue.

THERAPEUTIC METHODS AND COMPOSITIONS

The identification of PTAN as a normally testis-specific protein that is also expressed in cancers of the prostate and breast (and possibly other cancers), opens a number of therapeutic approaches to the treatment of such cancers. As discussed above, it is possible that PTAN functions as a transcription factor involved in activating tumor-promoting genes or repressing genes that block tumorigenesis.

Accordingly, therapeutic approaches aimed at inhibiting the activity of the PTAN protein are expected to be useful for patients suffering from prostate cancer, breast cancer, and other cancers expressing PTAN. These therapeutic approaches generally fall into two classes. One class comprises various methods for inhibiting the binding or association of the PTAN protein with its binding partner or with others proteins. Another class comprises a variety of methods for inhibiting the transcription of the PTAN gene or translation of PTAN mRNA.

A. THERAPEUTIC INHIBITION OF PTAN WITH INTRACELLULAR ANTIBODIES

Recombinant vectors encoding single chain antibodies which specifically bind to PTAN may be introduced into PTAN expressing cells via gene transfer technologies, wherein the encoded single chain anti-PTAN antibody is expressed intracellularly, binds to PTAN protein, and thereby inhibits its function. Methods for engineering such intracellular single chain antibodies are well known. Such intracellular antibodies, also known as "intrabodies", may be specifically targeted to a particular compartment within the cell, providing control over where the inhibitory activity of the treatment will be focused. This technology has been successfully applied in the art (for review, see Richardson and Marasco, 1995, TIBTECH vol. 13). Intrabodies have been shown to virtually eliminate the expression of otherwise abundant cell surface receptors. See, for example, Richardson et al., 1995, Proc. Natl. Acad. Sci. USA 92: 3137-3141; Beerli

et al., 1994, J. Biol. Chem. 289: 23931-23936; Deshane et al., 1994, Gene Ther. 1: 332-337.

Single chain antibodies comprise the variable domains of the heavy and light chain joined by a flexible linker polypeptide, and are expressed as a single polypeptide. Optionally, single chain antibodies may be expressed as a single chain variable region fragment joined to the light chain constant region. Well known intracellular trafficking signals may be engineered into recombinant polynucleotide vectors encoding such single chain antibodies in order to precisely target the expressed intrabody to the desired intracellular compartment. For example, intrabodies targeted to the endoplasmic reticulum (ER) may be engineered to incorporate a leader peptide and, optionally, a C-terminal ER retention signal, such as the KDEL amino acid motif. Intrabodies intended to exert activity in the nucleus may be engineered to include a nuclear localization signal. Lipid moieties may be joined to intrabodies in order to tether the intrabody to the cytosolic side of the plasma membrane. Intrabodies may also be targeted to exert function in the cytosol. For example, cytosolic intrabodies may be used to sequester factors within the cytosol, thereby preventing them from being transported to their natural cellular destination.

In one embodiment, intrabodies may be used to capture PTAN in the nucleus, thereby preventing its activity within the nucleus. Nuclear targeting signals may be engineered into such PTAN intrabodies in order to achieve the desired targeting. Such PTAN intrabodies may be designed to bind specifically to a particular PTAN domain, such as, for example, the bHLH domain of the PTAN protein. In another embodiment, cytosolic intrabodies which specifically bind to the PTAN protein may be used to prevent PTAN from gaining access to the nucleus, thereby preventing it from exerting any biological activity within the nucleus (e.g., preventing PTAN from forming transcription complexes with other factors).

In order to specifically direct the expression of such intrabodies to particular tumor cells, the transcription of the intrabody may be placed under the regulatory control of an appropriate tumor-specific promoter and/or enhancer. In order to target intrabody expression specifically to prostate, for example, the PSA promoter and/or promoter/enhancer may be utilized (See, for example, U.S. Patent No. 5,919,652).

B. THERAPEUTIC METHODS BASED ON INHIBITION OF PTAN TRANSCRIPTION OR TRANSLATION

Within the second class of therapeutic approaches, the invention provides various methods and compositions for inhibiting the transcription of the PTAN gene. Similarly, the invention also provides methods and compositions for inhibiting the translation of PTAN mRNA into protein.

In one approach, a method of inhibiting the transcription of the PTAN gene comprises contacting the PTAN gene with a PTAN antisense polynucleotide. In another approach, a method of inhibiting PTAN mRNA translation comprises contacting the PTAN mRNA with an antisense polynucleotide. In another approach, a PTAN specific ribozyme may be used to cleave the PTAN message, thereby inhibiting translation. Such antisense and ribozyme based methods may also be directed to the regulatory regions of the PTAN gene, such as the PTAN promoter and/or enhancer elements. Similarly, proteins capable of inhibiting a PTAN gene transcription factor may be used to inhibit PTAN mRNA transcription. The various polynucleotides and compositions useful in the aforementioned methods have been described above. The use of antisense and ribozyme molecules to inhibit transcription and translation is well known in the art.

Other factors which inhibit the transcription of PTAN through interfering with PTAN transcriptional activation may also be useful for the treatment of cancers expressing PTAN. Similarly, factors which are capable of interfering with PTAN processing may be useful for the treatment of cancers expressing PTAN. Cancer treatment methods utilizing such factors are also within the scope of the invention.

C. GENERAL CONSIDERATIONS

Gene transfer and gene therapy technologies may be used for delivering therapeutic polynucleotide molecules to tumor cells synthesizing PTAN (i.e., antisense, ribozyme, polynucleotides encoding intrabodies and other PTAN inhibitory molecules). A number of gene therapy approaches are known in the art. Recombinant vectors encoding PTAN antisense polynucleotides, ribozymes, factors capable of interfering with PTAN transcription, and so forth, may be delivered to target tumor cells using such gene therapy approaches.

The above therapeutic approaches may be combined with chemotherapy or radiation therapy regimens. These therapeutic approaches may also enable the use of reduced

dosages of chemotherapy and/or less frequent administration, particularly in patients that do not tolerate the toxicity of the chemotherapeutic agent well.

The anti-tumor activity of a particular composition (e.g., antisense, ribozyme, intrabody), or a combination of such compositions, may be evaluated using various in vitro and in vivo assay systems. In vitro assays for evaluating therapeutic potential include cell growth assays, soft agar assays and other assays indicative of tumor promoting activity, binding assays capable of determining the extent to which a therapeutic composition will inhibit the binding of PTAN to a binding partner, etc.

In vivo, the effect of a PTAN therapeutic composition may be evaluated in a suitable animal model. For example, xenogenic prostate cancer models wherein human prostate cancer explants or passaged xenograft tissues are introduced into immune compromised animals, such as nude or SCID mice, are appropriate in relation to prostate cancer and have been described (Klein et al., 1997, Nature Medicine 3: 402-408). For Example, PCT Patent Application WO98/16628, Sawyers et al., published April 23, 1998, describes various xenograft models of human prostate cancer capable of recapitulating the development of primary tumors, micrometastasis, and the formation of osteoblastic metastases characteristic of late stage disease. Efficacy may be predicted using assays which measure inhibition of tumor formation, tumor regression or metastasis, and the like. See, also, the Examples below.

In vivo assays which qualify the promotion of apoptosis may also be useful in evaluating potential therapeutic compositions. In one embodiment, xenografts from bearing mice treated with the therapeutic composition may be examined for the presence of apoptotic foci and compared to un-treated control xenograft-bearing mice. The extent to which apoptotic foci are found in the tumors of the treated mice provides an indication of the therapeutic efficacy of the composition.

The therapeutic compositions used in the practice of the foregoing methods may be formulated into pharmaceutical compositions comprising a carrier suitable for the desired delivery method. Suitable carriers include any material which when combined with the therapeutic composition retains the anti-tumor function of the therapeutic composition and is non-reactive with the patient's immune system. Examples include, but are not limited to, any of a number of standard pharmaceutical carriers such as sterile phosphate buffered saline solutions, bacteriostatic water, and the like (see, generally, Remington's Pharmaceutical Sciences 16th Edition, A. Osal., Ed., 1980).

Therapeutic formulations may be solubilized and administered via any route capable of delivering the therapeutic composition to the tumor site. Potentially effective routes of administration include, but are not limited to, intravenous, parenteral, intraperitoneal, intramuscular, intratumor, intradermal, intraorgan, orthotopic, and the like. A preferred formulation for intravenous injection comprises the therapeutic composition in a solution of preserved bacteriostatic water, sterile unpreserved water, and/or diluted in polyvinylchloride or polyethylene bags containing 0.9% sterile Sodium Chloride for Injection, USP. Therapeutic protein preparations may be lyophilized and stored as sterile powders, preferably under vacuum, and then reconstituted in bacteriostatic water containing, for example, benzyl alcohol preservative, or in sterile water prior to injection.

Dosages and administration protocols for the treatment of cancers using the foregoing methods will vary with the method and the target cancer and will generally depend on a number of other factors appreciated in the art.

CANCER VACCINES

The invention further provides prostate cancer vaccines comprising a PTAN protein or fragment thereof, as well as DNA based vaccines. In view of the testis-restricted expression of PTAN in normal human tissues (and the existence of the testis-blood barrier), PTAN cancer vaccines are expected to be effective at specifically preventing and/or treating PTAN expressing cancers without creating non-specific effects on non-target tissues. The use of a tumor antigen in a vaccine for generating humoral and cell-mediated immunity for use in anti-cancer therapy is well known in the art and has been employed in prostate cancer using human PSMA and rodent PAP immunogens (Hodge et al., 1995, Int. J. Cancer 63: 231-237; Fong et al., 1997, J. Immunol. 159: 3113-3117). Such methods can be readily practiced by employing a PTAN protein, or fragment thereof, or a PTAN-encoding nucleic acid molecule and recombinant vectors capable of expressing and appropriately presenting the PTAN immunogen.

For example, viral gene delivery systems may be used to deliver a PTAN-encoding nucleic acid molecule. Various viral gene delivery systems which can be used in the practice of this aspect of the invention include, but are not limited to, vaccinia, fowlpox, canarypox, adenovirus, influenza, poliovirus, adeno-associated virus, lentivirus, and sindbus virus (Restifo, 1996, Curr. Opin. Immunol. 8: 658-663). Non-viral delivery systems may also be employed by using naked DNA encoding a PTAN protein or fragment thereof introduced into the patient (e.g., intramuscularly) to induce an anti-tumor response. In one embodiment, the full-length human PTAN cDNA may be employed. In another embodiment, PTAN nucleic acid molecules encoding specific

cytotoxic T lymphocyte (CTL) epitopes may be employed. CTL epitopes can be determined using specific algorithms (e.g., Epimer, Brown University) to identify peptides within a PTAN protein which are capable of optimally binding to specified HLA alleles.

Various ex vivo strategies may also be employed. One approach involves the use of dendritic cells to present PTAN antigen to a patient's immune system. Dendritic cells express MHC class I and II, B7 costimulator, and IL-12, and are thus highly specialized antigen presenting cells. In prostate cancer, autologous dendritic cells pulsed with peptides of the prostate-specific membrane antigen (PSMA) are being used in a Phase I clinical trial to stimulate prostate cancer patients' immune systems (Tjoa et al., 1996, Prostate 28: 65-69; Murphy et al., 1996, Prostate 29: 371-380). Dendritic cells can be used to present PTAN peptides to T cells in the context of MHC class I and II molecules. In one embodiment, autologous dendritic cells are pulsed with PTAN peptides capable of binding to MHC molecules. In another embodiment, dendritic cells are pulsed with the complete PTAN protein. Yet another embodiment involves engineering the overexpression of the PTAN gene in dendritic cells using various implementing vectors known in the art, such as adenovirus (Arthur et al., 1997, Cancer Gene Ther. 4: 17-25), retrovirus (Henderson et al., 1996, Cancer Res. 56: 3763-3770), lentivirus, adeno-associated virus, DNA transfection (Ribas et al., 1997, Cancer Res. 57: 2865-2869), and tumor-derived RNA transfection (Ashley et al., 1997, J. Exp. Med. 186: 1177-1182). Cells expressing PTAN may also be engineered to express immune modulators, such as GM-CSF, and used as immunizing agents.

Anti-idiotypic anti-PTAN antibodies can also be used in anti-cancer therapy as a vaccine for inducing an immune response to cells expressing a PTAN protein. Specifically, the generation of anti-idiotypic antibodies is well known in the art and can readily be adapted to generate anti-idiotypic anti-PTAN antibodies that mimic an epitope on a PTAN protein (see, for example, Wagner et al., 1997, Hybridoma 16: 33-40; Foon et al., 1995, J Clin Invest 96: 334-342; Herlyn et al., 1996, Cancer Immunol Immunother 43: 65-76). Such an anti-idiotypic antibody can be used in cancer vaccine strategies.

Genetic immunization methods may be employed to generate prophylactic or therapeutic humoral and cellular immune responses directed against cancer cells expressing PTAN. Constructs comprising DNA encoding a PTAN protein/immunogen and appropriate regulatory sequences may be injected directly into muscle or skin of an individual, such that the cells of the muscle or skin take-up the construct and express the encoded PTAN protein/immunogen. Expression of the PTAN protein immunogen results

in the generation of prophylactic or therapeutic humoral and cellular immunity against prostate cancer. Various prophylactic and therapeutic genetic immunization techniques known in the art may be used (for review, see information and references published at Internet address www.genweb.com).

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KITS

For use in the diagnostic and therapeutic applications described or suggested above, kits are also provided by the invention. Such kits may comprise a carrier means being compartmentalized to receive in close confinement one or more container means such as vials, tubes, and the like, each of the container means comprising one of the separate elements to be used in the method. For example, one of the container means may comprise a probe which is or can be detectably labeled. Such probe may be an antibody or polynucleotide specific for a PTAN protein or a PTAN gene or message, respectively. Where the kit utilizes nucleic acid hybridization to detect the target nucleic acid, the kit may also have containers containing nucleotide(s) for amplification of the target nucleic acid sequence and/or a container comprising a reporter-means, such as a biotin-binding protein, such as avidin or streptavidin, bound to a reporter molecule, such as an enzymatic, florescent, or radioisotope label.

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EXAMPLES

Various aspects of the invention are further described and illustrated by way of the several examples which follow, none of which are intended to limit the scope of the invention.

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EXAMPLE 1:

SSH-GENERATED ISOLATION OF cDNA FRAGMENT OF THE PTAN GENE

MATERIALS AND METHODS

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LAPC Xenografts:

LAPC xenografts were obtained from Dr. Charles Sawyers (UCLA) and generated as described (Klein et al, 1997, Nature Med. 3: 402-408). Androgen dependent and independent LAPC-4 xenografts LAPC-4 AD and AI, respectively) and LAPC-9 AD xenografts were grown in male SCID mice and were passaged as small tissue chunks in recipient males. LAPC-4 AI xenografts were derived from LAPC-4 AD tumors. Male mice bearing LAPC-4 AD tumors were castrated and maintained for 2-3 months. After

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the LAPC-4 tumors re-grew, the tumors were harvested and passaged in castrated males or in female SCID mice.

Cell Lines:

Human cell lines (e.g., HeLa) were obtained from the ATCC and were maintained in DMEM with 5% fetal calf serum.

RNA Isolation:

Tumor tissue and cell lines were homogenized in Trizol reagent (Life Technologies, Gibco BRL) using 10 ml/ g tissue or 10 ml/ 10^8 cells to isolate total RNA. Poly A RNA was purified from total RNA using Qiagen's Oligotex mRNA Mini and Midi kits. Total and mRNA were quantified by spectrophotometric analysis (O.D. 260/280 nm) and analyzed by gel electrophoresis.

Oligonucleotides:

The following HPLC purified oligonucleotides were used.

DPNCDN (cDNA synthesis primer):

5'TTTTGATCAAGCTT$_{30}$3'

Adaptor 1:

5'CTAATACGACTCACTATAGGGCTCGAGCGGCCGCCCGGGCAG3'

3'GGCCCGTCCTAG5'

Adaptor 2:

5'GTAATACGACTCACTATAGGGCAGCGTGGTCGCGGCCGAG3'

3'CGGCTCCTAG5'

PCR primer 1:

5'CTAATACGACTCACTATAGGGC3'

Nested primer (NP)1:

5'TCGAGCGGCCGCCCGGGCAGGA3'

Nested primer (NP)2:

5'AGCGTGGTCGCGGCCGAGGA3'

Suppression Subtractive Hybridization:

Suppression Subtractive Hybridization (SSH) was used to identify cDNAs corresponding to genes which may be differentially expressed in prostate cancer. The

SSH reaction utilized cDNA from two different LAPC xenografts, subtracting LAPC-9 AD cDNA from LAPC-4 AD cDNA . The LAPC-4 AD xenograft was used as the source of the "tester" cDNA, while the LAPC-9 AD cDNA was used as the source of the "driver" cDNA.

Double stranded cDNAs corresponding to tester and driver cDNAs were synthesized from 2 μg of poly(A)$^+$ RNA isolated from the relevant xenograft tissue, as described above, using CLONTECH's PCR-Select cDNA Subtraction Kit and 1 ng of oligonucleotide DPNCDN as primer. First- and second-strand synthesis were carried out as described in the Kit's user manual protocol (CLONTECH Protocol No. PT1117-1, Catalog No. K1804-1). The resulting cDNA was digested with Dpn II for 3 hrs. at 37°C. Digested cDNA was extracted with phenol/chloroform (1:1) and ethanol precipitated.

Driver cDNA was generated by combining in a 1:1 ratio Dpn II digested cDNA from the relevant xenograft source (see above) with a mix of digested cDNAs derived from human benign prostatic hyperplasia (BPH), the human cell lines HeLa, 293, A431, Colo205, and mouse liver.

Tester cDNA was generated by diluting 1 μl of Dpn II digested cDNA from the relevant xenograft source (see above) (400 ng) in 5 μl of water. The diluted cDNA (2 μl, 160 ng) was then ligated to 2 μl of Adaptor 1 and Adaptor 2 (10 μM), in separate ligation reactions, in a total volume of 10 μl at 16°C overnight, using 400 u of T4 DNA ligase (CLONTECH). Ligation was terminated with 1 μl of 0.2 M EDTA and heating at 72°C for 5 min.

The first hybridization was performed by adding 1.5 μl (600 ng) of driver cDNA to each of two tubes containing 1.5 μl (20 ng) Adaptor 1- and Adaptor 2- ligated tester cDNA. In a final volume of 4 μl, the samples were overlaid with mineral oil, denatured in an MJ Research thermal cycler at 98°C for 1.5 minutes, and then were allowed to hybridize for 8 hrs at 68°C. The two hybridizations were then mixed together with an additional 1 μl of fresh denatured driver cDNA and were allowed to hybridize overnight at 68°C. The second hybridization was then diluted in 200 μl of 20 mM Hepes, pH 8.3, 50 mM NaCl, 0.2 mM EDTA, heated at 70°C for 7 min. and stored at -20°C.

<u>PCR Amplification, Cloning and Sequencing of Gene Fragments Generated from SSH</u>: To amplify gene fragments resulting from SSH reactions, two PCR amplifications were performed. In the primary PCR reaction 1 μl of the diluted final hybridization mix was

added to 1 µl of PCR primer 1 (10 µM), 0.5 µl dNTP mix (10 µM), 2.5 µl 10 x reaction buffer (CLONTECH) and 0.5 µl 50 x Advantage cDNA polymerase Mix (CLONTECH) in a final volume of 25 µl. PCR 1 was conducted using the following conditions: 75°C for 5 min., 94°C for 25 sec., then 27 cycles of 94°C for 10 sec, 66°C for 30 sec, 72°C for 1.5 min. Five separate primary PCR reactions were performed for each experiment. The products were pooled and diluted 1:10 with water. For the secondary PCR reaction, 1 µl from the pooled and diluted primary PCR reaction was added to the same reaction mix as used for PCR 1, except that primers NP1 and NP2 (10 µM) were used instead of PCR primer 1. PCR 2 was performed using 10-12 cycles of 94°C for 10 sec, 68°C for 30 sec, 72°C for 1.5 minutes. The PCR products were analyzed using 2% agarose gel electrophoresis.

The PCR products were inserted into pCR2.1 using the T/A vector cloning kit (Invitrogen). Transformed E. coli were subjected to blue/white and ampicillin selection. White colonies were picked and arrayed into 96 well plates and were grown in liquid culture overnight. To identify inserts, PCR amplification was performed on 1 ml of bacterial culture using the conditions of PCR1 and NP1 and NP2 as primers. PCR products were analyzed using 2% agarose gel electrophoresis.

Bacterial clones were stored in 20% glycerol in a 96 well format. Plasmid DNA was prepared, sequenced, and subjected to nucleic acid homology searches of the GenBank, dBest, and NCI-CGAP databases.

RT-PCR Expression Analysis:

First strand cDNAs were generated from 1 µg of mRNA with oligo (dT)12-18 priming using the Gibco-BRL Superscript Preamplification system. The manufacturers protocol was used and included an incubation for 50 min at 42°C with reverse transcriptase followed by RNAse H treatment at 37°C for 20 min. After completing the reaction, the volume was increased to 200 µl with water prior to normalization. First strand cDNAs from 16 different normal human tissues were obtained from Clontech.

Normalization of the first strand cDNAs from multiple tissues was performed by using the primers 5'atatcgccgcgctcgtcgtcgacaa3' and 5'agccacacgcagctcattgtagaagg 3' to amplify β-actin. First strand cDNA (5 µl) was amplified in a total volume of 50 µl containing 0.4 µM primers, 0.2 µM each dNTPs, 1XPCR buffer (Clontech, 10 mM Tris-HCL, 1.5 mM $MgCl_2$, 50 mM KCl, pH8.3) and 1X Klentaq DNA polymerase (Clontech). Five µl of the PCR reaction was removed at 18, 20, and 22 cycles and used

for agarose gel electrophoresis. PCR was performed using an MJ Research thermal cycler under the following conditions: initial denaturation was at 94°C for 15 sec, followed by a 18, 20, and 22 cycles of 94°C for 15, 65°C for 2 min, 72°C for 5 sec. A final extension at 72°C was carried out for 2 min. After agarose gel electrophoresis, the band intensities of the 283 bp β-actin bands from multiple tissues were compared by visual inspection. Dilution factors for the first strand cDNAs were calculated to result in equal β-actin band intensities in all tissues after 22 cycles of PCR. Three rounds of normalization were required to achieve equal band intensities in all tissues after 22 cycles of PCR.

To determine expression levels of the PTAN gene, 5 μl of normalized first strand cDNA was analyzed by PCR using 25, 30, and 35 cycles of amplification using the following primer pairs, which were designed with the assistance of (MIT; for details, see, www.genome.wi.mit.edu):

5' - TTG CAG TAG ATA GGT GGT CAG CTC C – 3'
5' - CAA AGC AGA ATG TTT CAC CTC CA – 3'

Semi quantitative expression analysis was achieved by comparing the PCR products at cycle numbers that give light band intensities.

RESULTS:

Two SSH experiments described in the Materials and Methods, supra, led to the isolation of numerous candidate gene fragment clones (SSH clones). All candidate clones were sequenced and subjected to homology analysis against all sequences in the major public gene and EST databases in order to provide information on the identity of the corresponding gene and to help guide the decision to analyze a particular gene for differential expression. In general, gene fragments which had no homology to any known sequence in any of the searched databases, and thus considered to represent novel genes, as well as gene fragments showing homology to previously sequenced expressed sequence tags (ESTs), were subjected to differential expression analysis by RT-PCR and/or Northern analysis.

One of the SHH clones comprising about 466 bp, showed significant homology to several testis-derived ESTs but no homology to any known gene, and was designated 26P5C7. The 26P5C7 sequence encodes a 125 amino acid open reading frame in its 5'

end. The nucleotide and deduced ORF sequences of this SHH clone are shown in FIG. 4.

Differential expression analysis by RT-PCR showed some degree of over-expression in the LAPC-4 xenografts relative to normal prostate (FIG. 5A, Panel A). In addition, RT-PCR expression analysis of first strand cDNAs from 16 normal tissues showed a relatively testis-specific expression pattern in adult tissues, with lower level expression detectable in placenta, at 30 cycles of amplification.

EXAMPLE 2: FULL LENGTH CLONING OF PTAN-1 AND PTAN-2

Full length cDNAs encoding two isoforms of the 26P5C7 gene were subsequently isolated from a testis library and designated PTAN-1 and PTAN-2. The nucleotide and amino acid sequences of PTAN-1 (GTD2) and PTAN-2 (GTC6) are shown in FIGS 1 and 2, respectively. The open reading frames (ORFs) of PTAN-1 and PTAN-2 are identical with the exception of a 31 amino acid insertion at residues 45-76 in PTAN-1, resulting in a 434 amino acid ORF compared to a 403 amino acid ORF for PTAN-2. The PTAN-1 and PTAN-2 cDNAs were deposited on November 5, 1998 with the American Type Culture Collection (ATCC; Manassas, VA) as plasmids p26P5C7-GTD2 and p26P5C7-GTC6, respectively, and have been assigned Accession Nos. 98976 and 98977, respectively.

To confirm the presence of the 31 residue insert in PTAN-1, RT-PCR was performed on 1st strand cDNA derived from the LAPC-4 AD xenograft. PCR primers were designed within and outside of the insert region. The RT-PCR result confirmed that a cDNA containing the insert exists in the xenograft. Both PTAN-1 and PTAN-2 exhibit a putative nuclear localization signal at the amino terminus (residues 20-26). Using the PSORT program, the PTAN proteins are predicted to be nuclear in localization. The PTAN proteins have no homology to any known proteins, but the sequence does overlap with several ESTs derived from testis.

EXAMPLE 3: FULL LENGTH CLONING OF PTAN-3

A full length cDNA encoding a third isoform of the 26P5C7 gene was isolated from a testis library and designated PTAN-3. The nucleotide and amino acid sequences of PTAN-3 (26P5C7-GTP1C8) are shown in FIG. 3. The PTAN-3 cDNA was deposited as plasmid p26P5C7-GTP1C8 with the American Type Culture Collection (ATCC;

Manassas, VA) on February 12, 1999 as Accession No. 207095. An alignment of the amino acid sequences encoded by PTAN-1, PTAN-2, and PTAN-3 cDNAs (FIGS. 1, 2, and 3, respectively) is shown in FIG. 4. PTAN-3 is substantially identical to PTAN-1, except that it is truncated at the amino terminus (i.e., missing the first 56 amino acids of the PTAN-1 structure) and contains a 3 amino acid gap.

EXAMPLE 4:

PTAN GENE EXPRESSION ANALYSIS – TESTIS SPECIFIC IN NORMAL TISSUES

PTAN mRNA expression in normal human tissues was first analyzed by Northern blotting of two multiple tissue blots (Clontech; Palo Alto, California), comprising a total of 16 different normal human tissues, using labeled 26P5C7 SSH fragment (Example 1) as a probe. RNA samples were quantitatively normalized with a β–actin probe. The results of this analysis are shown in FIG. 9. Expression of a 3 kb transcript was only detected in normal testis.

PTAN expression in normal tissues was further analyzed using a multi-tissue RNA dot blot containing 76 different samples (representing mainly normal tissues as well as a few cancer cell lines) demonstrated strong expression of PTAN only in testis (FIG. 10).

EXAMPLE 5:

PTAN EXPRESSION IN PROSTATE CANCER AND OTHER CANCERS

To analyze PTAN expression in cancer tissues and cell lines, Northern blot analysis was performed on RNA derived from the LAPC prostate cancer xenografts as well as a panel of prostate cancer and other cancer cell lines. The results (FIG. 11) show high levels of PTAN expression in LAPC-4 AD and LAPC-4 AI, with lower levels detected several cancer cell lines derived from breast (BT-20, DU4475). These results suggest that PTAN is a very testis specific gene that is up-regulated in prostate cancer and in breast cancer.

EXAMPLE 6:

GENERATION OF PTAN POLYCLONAL ANTIBODIES

To generate polyclonal antibodies directed against PTAN a 15 mer peptide was designed from a coding region common to all PTAN isoforms. The peptide CQAASDSSHKIPISN was conjugated to keyhole limpet hemocyanin (KLH) and was used to immunize a rabbit.

To test the rabbit serum for reactivity with PTAN proteins, full length PTAN-1 and PTAN-2 cDNAs were cloned into an expression vector that provides a 6His tag at the carboxyl-terminus (pCDNA 3.1 myc-his, InVitrogen). After transfection of the constructs into 293T cells, cell lysates were probed with anti-His antibody (Santa Cruz) and the anti-PTAN serum using Western blotting. Anti-His western blotting clearly shows expression of both PTAN-1 and PTAN-2, which migrate at the predicted molecular weights of 49 and 46 Kilodaltons (KD) respectively (FIG. 12).

Even though both gene isoforms were expressed using the same vector, expression of PTAN-1 was 10-20 fold higher than expression of PTAN-2. It is possible that PTAN-2 is less stable than PTAN-1 protein and that the additional sequence in PTAN-1 confers protein stability. The anti-PTAN antibody only recognized PTAN-1, even though the immunogen peptide sequence is present in both isoforms. This is probably due to the lower sensitivity of PTAN antibodies compared to anti-His and the lower expression level of PTAN-2. This polyclonal serum is specific for PTAN and may be used to assess the expression of PTAN in patient samples.

EXAMPLE 7:

PRODUCTION OF RECOMBINANT PTAN IN A MAMMALIAN SYSTEMS

To express recombinant PTAN-1 and PTAN-2, the full length PTAN-1 and PTAN-2 cDNAs were separately cloned into an expression vector that provides a 6His tag at the carboxyl-terminus (pCDNA 3.1 myc-his, InVitrogen). The constructs was transfected into 293T cells. Transfected 293T cell lysates were probed with the anti-PTAN polyclonal serum described in Example 5 above in a Western blot. The results show that the polyclonal serum recognizes a 49 and 46 kilodalton (KD) proteins only in the PTAN-1 and PTAN-2 transfected cells, respectively, and not in the control vector transfected cells (FIG. 12).

The PTAN genes were subcloned into the retroviral expression vector pSRαMSVtkneo and used to establish PTAN expressing cell lines as follows. The PTAN coding sequence (from translation initiation ATG to the termination codons) was amplified by PCR using ds cDNA template from PTAN cDNA. The PCR product was subcloned into pSRαMSVtkneo via the EcoR1(blunt-ended) and Xba 1 restriction sites on the vector and transformed into DH5α competent cells. Colonies were picked to screen for clones with unique internal restriction sites on the cDNA. The positive clone was

confirmed by sequencing of the cDNA insert. Retroviruses were used for infection and generation of various cell lines using, for example, 3T3CL7, PC3, and LnCap cells.

EXAMPLE 8:

PRODUCTION OF RECOMBINANT PTAN IN A BACULOVIRUS SYSTEM

To generate a recombinant PTAN protein in a baculovirus expression system, the PTAN cDNA is cloned into the baculovirus transfer vector pBlueBac 4.5 (Invitrogen) which provides a His-tag at the N-terminus Specifically, pBlueBac--PTAN is co-transfected with helper plasmid pBac-N-Blue (Invitrogen) into SF9 (Spodoptera frugiperda) insect cells to generate recombinant baculovirus (see Invitrogen instruction manual for details). Baculovirus is then collected from cell supernatant and purified by plaque assay.

Recombinant PTAN protein is then generated by infection of HighFive insect cells (InVitrogen) with the purified baculovirus. Recombinant PTAN protein may be detected using anti-PTAN antibody. PTAN protein may be purified and used in various cell based assays or as immunogen to generate polyclonal and monoclonal antibodies specific for PTAN.

EXAMPLE 9:

CHROMOSOMAL MAPPING OF THE PTAN GENE

The chromosomal localization of PTAN was determined using the GeneBridge4 radiation hybrid panel (Walter et al., 1994, Nat. Genetics 7:22) (Research Genetics, Huntsville Al). The following PCR primers were used to localize PTAN:

1. TCC AAT TCT TCC AGC AAT ATC CAC
2. AGG AAG TTG GGC CTG TTA CTG TTT

The resulting mapping vector for the 93 radiation hybrid panel DNAs was:
00010100010011000000000001000000110000101010100000010100000000100000000101100000001000000000001

This vector and the mapping program at http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl placed PTAN on chromosome 1q22.

EXAMPLE 10:

IDENTIFICATION OF POTENTIAL SIGNAL TRANSDUCTION PATHWAYS

To determine whether PTAN directly or indirectly activates known signal transduction pathways in cells, luciferase (luc) based transcriptional reporter assays are carried out in cells expressing PTAN. These transcriptional reporters contain consensus binding sites for known transcription factors which lie downstream of well characterized signal transduction pathways. The reporters and examples of there associated transcription factors, signal transduction pathways, and activation stimuli are listed below.

1. NFkB-luc, NFkB/Rel; Ik-kinase/SAPK; growth/apoptosis/stress
2. SRE-luc, SRF/TCF/ELK1; MAPK/SAPK; growth/differentiation
3. AP-1-luc, FOS/JUN; MAPK/SAPK/PKC; growth/apoptosis/stress
4. ARE-luc, androgen receptor; steroids/MAPK; growth/differentiation/apoptosis
5. p53-luc, p53; SAPK; growth/differentiation/apoptosis
6. CRE-luc, CREB/ATF2; PKA/p38; growth/apoptosis/stress

PTAN-mediated effects may be assayed in cells showing mRNA expression. Luciferase reporter plasmids may be introduced by lipid mediated transfection (TFX-50, Promega). Luciferase activity, an indicator of relative transcriptional activity, is measured by incubation of cells extracts with luciferin substrate and luminescence of the reaction is monitored in a luminometer.

EXAMPLE 11:

GENERATION OF PTAN MONOCLONAL ANTIBODIES

In order to generate PTAN monoclonal antibodies, a glutathione-S-transferase (GST) fusion protein encompassing a PTAN protein is synthesized and used as immunogen. Balb C mice are initially immunized intraperitoneally with 200 μg of the GST-PTAN fusion protein mixed in complete Freund's adjuvant. Mice are subsequently immunized every 2 weeks with 75 μg of GST-PTAN protein mixed in Freund's incomplete adjuvant for a total of 3 immunizations. Reactivity of serum from immunized mice to full length PTAN protein is monitored by ELISA using a partially purified preparation of HIS-tagged PTAN protein expressed from 293T cells (Example 6). Mice showing the strongest reactivity are rested for 3 weeks and given a final injection of fusion protein in PBS and then sacrificed 4 days later. The spleens of the sacrificed mice are then harvested and fused to SPO/2 myeloma cells using standard procedures (Harlow and

Lane, 1988). Supernatants from growth wells following HAT selection are screened by ELISA and Western blot to identify PTAN specific antibody producing clones.

The binding affinity of a PTAN monoclonal antibody may be determined using standard technology. Affinity measurements quantify the strength of antibody to epitope binding and may be used to help define which PTAN monoclonal antibodies are preferred for diagnostic or therapeutic use. The BIAcore system (Uppsala, Sweden) is a preferred method for determining binding affinity. The BIAcore system uses surface plasmon resonance (SPR, Welford K. 1991, Opt. Quant. Elect. 23:1; Morton and Myszka, 1998, Methods in Enzymology 295: 268) to monitor biomolecular interactions in real time. BIAcore analysis conveniently generates association rate constants, dissociation rate constants, equilibrium dissociation constants, and affinity constants.

EXAMPLE 12:

IN VITRO ASSAYS OF PTAN FUNCTION

The expression of PTAN in prostate cancer and other cancers suggests a functional role in tumor progression. It is possible that PTAN functions as a transcription factor involved in activating genes involved in tumorigenesis or repressing genes that block tumorigenesis. PTAN function can be assessed in mammalian cells using in vitro approaches. For mammalian expression, PTAN can be cloned into a number of appropriate vectors, including pcDNA 3.1 myc-His-tag (Example 6) and the retroviral vector pSRαtkneo (Muller et al., 1991, MCB 11:1785). Using such expression vectors, PTAN can be expressed in several cell lines, including PC-3, NIH 3T3, LNCaP and 293T. Expression of PTAN can be monitored using anti-PTAN antibodies (see Examples 5 and 10).

Mammalian cell lines expressing PTAN can be tested in several in vitro and in vivo assays, including cell proliferation in tissue culture, activation of apoptotic signals, tumor formation in SCID mice, and in vitro invasion using a membrane invasion culture system (MICS) (Welch et al. ,Int. J. Cancer 43: 449-457). PTAN cell phenotype is compared to the phenotype of cells that lack expression of PTAN.

Cell lines expressing PTAN can also be assayed for alteration of invasive and migratory properties by measuring passage of cells through a matrigel coated porous membrane chamber (Becton Dickinson). Passage of cells through the membrane to the opposite side is monitored using a fluorescent assay (Becton Dickinson Technical

Bulletin #428) using calcein-Am (Molecular Probes) loaded indicator cells. Cell lines analyzed include parental and PTAN overexpressing PC3, 3T3 and LNCaP cells. To assay whether PTAN has chemoattractant properties, parental indicator cells are monitored for passage through the porous membrane toward a gradient of PTAN conditioned media compared to control media. This assay may also be used to qualify and quantify specific neutralization of the PTAN induced effect by candidate cancer therapeutic compositions.

EXAMPLE 13:

IN VIVO ASSAY FOR PTAN TUMOR GROWTH PROMOTION

The effect of the PTAN protein on tumor cell growth may be evaluated in vivo by gene overexpression in tumor-bearing mice. For example, SCID mice can be injected SQ on each flank with 1 x 10^6 of either PC3, TSUPR1, or DU145 cells containing tkNeo empty vector or PTAN. At least two strategies may be used: (1) Constitutive PTAN expression under regulation of an LTR promoter, and (2) Regulated expression under control of an inducible vector system, such as ecdysone, tet, etc. Tumor volume is then monitored at the appearance of palpable tumors and followed over time to determine if PTAN expressing cells grow at a faster rate. Additionally, mice may be implanted with 1 x 10^5 of the same cells orthotopically to determine if PTAN has an effect on local growth in the prostate or on the ability of the cells to metastasize, specifically to lungs, lymph nodes, and bone marrow.

The assay is also useful to determine the PTAN inhibitory effect of candidate therapeutic compositions, such as for example, PTAN intrabodies, PTAN antisense molecules and ribozymes.

EXAMPLE 14:

WESTERN ANALYSIS OF PTAN EXPRESSION IN SUBCELLULAR FRACTIONS

To determine the subcellular localization of PTAN, 293T cells were transfected with an expression vector encoding HIS-tagged PTAN (PCDNA 3.1 MYC/HIS, Invitrogen). The transfected cells were harvested and subjected to a differential subcellular fractionation protocol as previously described (Pemberton, P.A. et al, 1997, J of Histochemistry and Cytochemistry, 45:1697-1706.) This protocol separates the cell into fractions enriched for nuclei, heavy membranes (lysosomes, peroxisomes, and mitochondria), light membranes (plasma membrane and endoplasmic reticulum), and soluble proteins.

Western blot analysis of these fractions with either anti-HIS antibody or with anti-PTAN polyclonal antibody as probe demonstrates that PTAN localizes to the nuclear and heavy membrane fraction when overexpressed in 293T cells (FIG. 13). The blots were developed with species-specific HRP conjugated secondary Ab and visualized by enhanced chemiluminescense. The transfected cells were lysed in 2 mls of buffer and each subcellular fraction lane represents approximately 1/60th (by volume) of the starting material. The whole cell lysate lane represents approximately 1/160th of the starting material. Western analysis of the same subcellular fractions with antibodies to the nuclear proteins Cdk2 an c-Abl demonstrate co-localization of PTAN with these proteins to the same subcellular fractions.

This application claims the benefit of the filing dates of United States Provisional Patent Applications 06/129,518 filed 14 April 1998, 06/113,229 filed 21 December 1998 and 06/102,910 filed 2 October 1998, and 60/102,556 filed 30 September 1998 under the provisions of 37 USC 119(e), the contents of which are incorporated by reference herein in their entireties.

Throughout this application, various publications are referenced within parentheses. The disclosures of these publications are hereby incorporated by reference herein in their entireties.

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any which are functionally equivalent are within the scope of the invention. Various modifications to the models and methods of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

CLAIMS:

1. An isolated PTAN protein selected from the group consisting of (a) PTAN-1, having the amino acid sequence as shown in FIG. 1; (b) PTAN-2, having the amino acid sequence as shown in FIG. 2, and (3) PTAN-3, having the amino acid sequence as shown in FIG. 3.

2. An isolated polypeptide of at least 15 contiguous amino acids of the protein of claim 1.

3. An isolated polypeptide which is at least 90% identical to the amino acid sequence of the protein of claim 1 over its entire length.

4. An isolated polypeptide of at least 15 contiguous amino acids of the protein of claim 3.

5. An isolated polynucleotide selected from the group consisting of (a) a polynucleotide having the sequence as shown in FIG. 1, wherein T can also be U; (b) a polynucleotide having the sequence as shown in FIG. 1, from nucleotide residue number 26 through nucleotide residue number 1327, wherein T can also be U; (c) a polynucleotide having the sequence as shown in FIG. 2, wherein T can also be U; (d) a polynucleotide having the sequence as shown in FIG. 2, from nucleotide residue number 61 through nucleotide residue number 1269, wherein T can also be U; (e) a polynucleotide having the sequence as shown in FIG. 3, wherein T can also be U; (f) a polynucleotide having the sequence as shown in FIG. 3, from nucleotide residue number 271 through nucleotide residue number 1395, wherein T can also be U; (g) a polynucleotide encoding a PTAN polypeptide whose sequence is encoded by the cDNAs contained in the plasmids deposited with American Type Culture Collection as Accession Nos. 98976, 98977, and 207095; and (h) a polynucleotide encoding the PTAN protein of claim 1.

6. An isolated polynucleotide which selectively hybridizes under stringent conditions to a polynucleotide according to claim 5.

7. An isolated fragment of a polynucleotide according to claim 5 which is at least 20 nucleotide bases in length.

8. An isolated polynucleotide which is fully complementary to a polynucleotide according to claim 5.

9. A recombinant expression vector which contains a polynucleotide according to claim 5.

10. A host cell which contains an expression vector according to claim 9.

11. An isolated polynucleotide according to claim 5, 6, 7 or 8 which is labeled with a detectable marker.

12. A process for producing a PTAN protein comprising culturing a host cell of claim 10 under conditions sufficient for the production of the polypeptide and recovering the PTAN protein from the culture.

13. A PTAN polypeptide produced by the process of claim 12.

14. An antibody which specifically binds to the PTAN protein of claim 1.

15. A monoclonal antibody according to claim 14.

16. A monoclonal antibody according to claim 15 which is labeled with a detectable marker.

17. A monoclonal antibody according to claim 16, wherein the detectable marker is selected from the group consisting of a radioisotope, fluorescent compound, bioluminescent compound, chemiluminescent compound, metal chelator or enzyme.

18. An Fab, F(ab')2, Fv or Sfv fragment of a monoclonal antibody according to claim 15.

19. A fragment of a monoclonal antibody according to claim 18 which is labeled with a detectable marker.

20. A fragment of a monoclonal antibody according to claim 19, wherein the detectable marker is selected from the group consisting of a radioisotope,

fluorescent compound, bioluminescent compound, chemiluminescent compound, metal chelator or enzyme.

21. A monoclonal antibody according to claim 15 which comprises murine antigen binding region residues and human antibody residues.

22. A monoclonal antibody according to claim 15 which is a human antibody.

23. A transgenic animal producing a monoclonal antibody according to claim 22.

24. A hybridoma producing a monoclonal antibody according to claim 15.

25. A recombinant protein comprising the antigen binding region of a monoclonal antibody according to claim 15.

26. A recombinant protein according to claim 25 which is labeled with a detectable marker.

27. A recombinant protein according to claim 26, wherein the detectable marker is selected from the group consisting of a radioisotope, fluorescent compound, bioluminescent compound, chemiluminescent compound, metal chelator or enzyme.

28. A single chain monoclonal antibody which comprises the variable domains of the heavy and light chains of a monoclonal antibody according to claim 15.

29. A vector comprising a polynucleotide encoding a single chain monoclonal antibody according to claim 28.

30. An assay for detecting the presence of a PTAN protein in a biological sample comprising contacting the sample with an antibody of claim 16, an antibody fragment of claim 19, or a recombinant protein of claim 26, and detecting the binding of PTAN protein in the sample thereto.

31. An assay for detecting the presence of a PTAN polynucleotide in a biological sample, comprising

(a) contacting the sample with a polynucleotide probe which specifically hybridizes to the PTAN cDNA contained within the plasmid as deposited with American Type Culture Collection as Accession No. 98976, the plasmid as deposited with American Type Culture Collection as Accession No. 98977, the plasmid as deposited with American Type Culture Collection as Accession No. 207095, the polynucleotide as shown in FIG. 1, the polynucleotide as shown in FIG. 2, the polynucleotide as shown in FIG. 3, or the complements thereof; and

(b) detecting the presence of a hybridization complex formed by the hybridization of the probe with PTAN polynucleotide in the sample, wherein the presence of the hybridization complex indicates the presence of PTAN polynucleotide within the sample.

32. An assay for detecting the presence of PTAN mRNA in a biological sample comprising:

(a) producing cDNA from the sample by reverse transcription using at least one primer;

(b) amplifying the cDNA so produced using PTAN polynucleotides as sense and antisense primers to amplify PTAN cDNAs therein;

(c) detecting the presence of the amplified PTAN cDNA,

wherein the PTAN polynucleotides used as the sense and antisense probes are capable of amplifying the PTAN cDNA contained within the plasmid as deposited with American Type Culture Collection as Accession No. 98976, the plasmid as deposited with American Type Culture Collection as Accession No. 98977, or the plasmid as deposited with American Type Culture Collection as Accession No. 207095.

33. A method of detecting the presence of a cancer expressing PTAN protein which comprises determining the level of PTAN protein expressed by cells in a test tissue sample from an individual and comparing the level so determined to the level of PTAN expressed in a corresponding normal sample, the presence of elevated PTAN protein in the test sample relative to the normal sample providing an indication of the presence of such cancer in the individual.

34. A method of diagnosing the presence of cancer in an individual comprising:

(a) obtaining a test sample of tissue from the individual;

(b) determining the level of PTAN mRNA expressed in the test sample;

(c) comparing the level so determined to the level of PTAN mRNA expressed in a comparable known normal tissue sample,

the presence of elevated PTAN mRNA expression in the test sample relative to the normal tissue sample providing an indication of the presence of cancer.

35. The method of claim 34, wherein the cancer is prostate cancer, and the test and normal tissue samples are selected from the group consisting of prostate tissue, bone tissue, lymphatic tissue, serum, blood or semen.

36. The method of claim 34, wherein the cancer is breast cancer, and the test and normal tissue samples are selected from the group consisting of breast tissue, lymphatic tissue, serum, blood or urine.

37. A method of diagnosing the presence of cancer in an individual comprising:

(a) obtaining a test sample of tissue from the individual;

(b) determining the level of PTAN protein expressed in the test sample;

(c) comparing the level so determined to the level of PTAN protein expressed in a comparable known normal tissue sample,

the presence of elevated PTAN protein in the test sample relative to the normal tissue sample providing an indication of the presence of cancer.

38. The method of claim 37, wherein the cancer is prostate cancer, and the test and normal tissue samples are selected from the group consisting of prostate tissue, bone tissue, lymphatic tissue, serum, blood or semen.

39. The method of claim 37, wherein the cancer is breast cancer, and the test and normal tissue samples are selected from the group consisting of breast tissue, lymphatic tissue, serum, blood or urine.

40. A method of treating a patient with a cancer that expresses PTAN which comprises administering to said patient a vector according to claim 29, such that the vector delivers the single chain monoclonal antibody coding sequence to the cancer cells and the encoded single chain antibody is expressed intracellularly therein.

41. The method according to claim 40, wherein the cancer is selected from the group consisting of cancer of the prostate or breast.

42. A method of treating a patient with a cancer that expresses PTAN which comprises inhibiting the transcription of PTAN in the cells of said cancer.

43. The method according to claim 42, wherein PTAN transcription is inhibited by contacting the PTAN gene with an antisense polynucleotide complementary to a polynucleotide of claim 5.

44. A method of treating a patient with a cancer that expresses PTAN which comprises inhibiting the translation of PTAN mRNA in the cells of said cancer.

45. The method according to claim 44, wherein PTAN mRNA translation is inhibited by contacting the PTAN mRNA with an antisense polynucleotide complementary to a polynucleotide of claim 5.

46. The method according to claim 44, wherein PTAN mRNA translation is inhibited by contacting the PTAN mRNA with a ribozyme capable of cleaving said PTAN mRNA.

47. A vaccine composition for the treatment of a cancer expressing PTAN comprising a PTAN protein according to claim 1 and a physiologically acceptable carrier.

48. A vaccine composition for the treatment of a cancer expressing PTAN comprising an immunogenic portion of a PTAN protein according to claim 2 and a physiologically acceptable carrier.

49. A method of inhibiting the development of a cancer expressing PTAN in a patient, comprising administering to the patient an effective amount of the vaccine composition of claim 47 or 48.

FIG. 1

```
          10         19         28         37         46         55
5'  GCC AGG AAG TTT GAC CGC GCT GCC ATG CCG AAC CGT AAG GCC AGC CGG AAT GCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   R   K   F   D   R   A   A   M   P   N   R   K   A   S   R   N   A

          64         73         82         91        100        109
    TAC TAT TTC TTC GTG CAG GAG AAG ATC CCC GAA CTA CGG CGA CGA GGC CTG CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Y   Y   F   F   V   Q   E   K   I   P   E   L   R   R   R   G   L   P

         118        127        136        145        154        163
    GTG GCT CGC GTT GCT GAT GCC ATC CCT TAC TGC TCC TCA GAC TGG GCG CTT CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     V   A   R   V   A   D   A   I   P   Y   C   S   S   D   W   A   L   L

         172        181        190        199        208        217
    AGG GAG GAA GAA AAG GAG AAA TAC GCA GAA ATG GCT CGA GAA TGG AGG GCC GCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   E   E   E   K   E   K   Y   A   E   M   A   R   E   W   R   A   A

         226        235        244        253        262        271
    CAG GGA AAG GAC CCT GGG CCC TCA GAG AAG CAG AAA CCT GTT TTC ACA CCA CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Q   G   K   D   P   G   P   S   E   K   Q   K   P   V   F   T   P   L

         280        289        298        307        316        325
    AGG AGG CCA GGC ATG CTT GTA CCA AAG CAG AAT GTT TCA CCT CCA GAT ATG TCA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   R   P   G   M   L   V   P   K   Q   N   V   S   P   P   D   M   S

         334        343        352        361        370        379
    GCT TTG TCT TTA AAA GGT GAT CAA GCT CTC CTT GGA GGC ATT TTT TAT TTT TTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   L   S   L   K   G   D   Q   A   L   L   G   G   I   F   Y   F   L

         388        397        406        415        424        433
    AAC ATT TTT AGC CAT GGC GAG CTA CCT CCT CAT TGT GAA CAG CGC TTC CTC CCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     N   I   F   S   H   G   E   L   P   P   H   C   E   Q   R   F   L   P

         442        451        460        469        478        487
    TGT GAA ATT GGC TGT GTT AAG TAT TCT CTC CAA GAA GGT ATT ATG GCA GAT TTC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     C   E   I   G   C   V   K   Y   S   L   Q   E   G   I   M   A   D   F

         496        505        514        523        532        541
    CAC AGT TTT ATA AAT CCT GGT GAA ATT CCA CGA GGA TTT CGA TTT CAT TGT CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     H   S   F   I   N   P   G   E   I   P   R   G   F   R   F   H   C   Q

         550        559        568        577        586        595
    GCT GCA AGT GAT TCT AGT CAC AAG ATT CCT ATT TCA AAT TTT GAA CGT GGG CAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   A   S   D   S   S   H   K   I   P   I   S   N   F   E   R   G   H

         604        613        622        631        640        649
    AAC CAA GCA ACT GTG TTA CAA AAC CTT TAT AGA TTT ATT CAT CCC AAC CCA GGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     N   Q   A   T   V   L   Q   N   L   Y   R   F   I   H   P   N   P   G

         658        667        676        685        694        703
    AAC TGG CCA CCT ATC TAC TGC AAG TCT GAT GAT AGA ACC AGA GTC AAC TGG TGT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     N   W   P   P   I   Y   C   K   S   D   D   R   T   R   V   N   W   C

         712        721        730        739        748        757
    TTG AAG CAT ATG GCA AAG GCA TCA GAA ATC AGG CAA GAT CTA CAA CTT CTC ACT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   K   H   M   A   K   A   S   E   I   R   Q   D   L   Q   L   L   T
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       766        775        784        793        802        811
GTA GAG GAC CTT GTA GTG GGG ATC TAC CAA CAA AAA TTT CTC AAG GAG CCC TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 V   E   D   L   V   V   G   I   Y   Q   Q   K   F   L   K   E   P   S

       820        829        838        847        856        865
AAG ACT TGG ATT CGA AGC CTC CTA GAT GTG GCC ATG TGG GAT TAT TCT AGC AAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   T   W   I   R   S   L   L   D   V   A   M   W   D   Y   S   S   N

       874        883        892        901        910        919
ACA AGG TGC AAG TGG CAT GAA GAA AAT GAT ATT CTC TTC TGT GCT TTA GCT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   R   C   K   W   H   E   E   N   D   I   L   F   C   A   L   A   V

       928        937        946        955        964        973
TGC AAG AAG ATT GCG TAC TGC ATC AGT AAT TCT CTG GCC ACT CTC TTT GGA ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   K   K   I   A   Y   C   I   S   N   S   L   A   T   L   F   G   I

       982        991       1000       1009       1018       1027
CAG CTC ACA GAG GCT CAT GTA CCA CTA CAA GAT TAT GAG GCC AGC AAT AGT GTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   L   T   E   A   H   V   P   L   Q   D   Y   E   A   S   N   S   V

      1036       1045       1054       1063       1072       1081
ACA CCC AAA ATG GTT GTA TTG GAT GCA GGG CGT TAC CAG AAG CTA AGG GTT GGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   P   K   M   V   V   L   D   A   G   R   Y   Q   K   L   R   V   G

      1090       1099       1108       1117       1126       1135
AGT TCA GGA TTC TCT CAT TTC AAC TCT TCT AAT GAG GAA CAA AGA TCA AAC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   S   G   F   S   H   F   N   S   S   N   E   E   Q   R   S   N   T

      1144       1153       1162       1171       1180       1189
CCC ATT GGT GAC TAC CCA TCT AGG GCA AAA ATT TCT GGC CAA AAC AGC AGC GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   I   G   D   Y   P   S   R   A   K   I   S   G   Q   N   S   S   V

      1198       1207       1216       1225       1234       1243
CGG GGA AGA GGA ATT ACC CGC TTA CTA GAG AGC ATT TCC AAT TCT TCC AGC AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   G   R   G   I   T   R   L   L   E   S   I   S   N   S   S   S   N

      1252       1261       1270       1279       1288       1297
ATC CAC AAA TTC TCC AAC TGT GAC ACT TCA CTC TCA CCT TAC ATG TCC CAA AAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   H   K   F   S   N   C   D   T   S   L   S   P   Y   M   S   Q   K

      1306       1315       1324       1333       1342       1351
GAT GGA TAC AAA TCT TTC TCT TCC TTA TCT TAA TGA TGG TAC TCT TTT CAA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   G   Y   K   S   F   S   S   L   S   *   *

      1360       1369       1378       1387       1396       1405
CTG AAA ACA GTA ACA GGC CCA ACT TCC TTC TTA CTA CAG TCA TAT TAA ACA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1414       1423       1432       1441       1450       1459
CAC ATC AAT GAC AAA TGT CAC TAC TAT AAA AAC TAC TTA ATT TGT AAG GAA ATT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1468       1477       1486       1495       1504       1513
GTT TCA TAG ATT TAA AAA AAT TGT GGT TGG AGA GCA TCT TGG CAT TTG TGC TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1522       1531       1540       1549       1558       1567
TTT TCT TGA GGG ATT GTT CTG CTT CCT GGC TGT ATG ATG GGT ATA TCA TTA AAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1576       1585       1594       1603       1612       1621
TTT GGA GTC CTA TAT GAA CAA AAC TGA CAT TTT TAG AGT TGT ACT TTT GGG AAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1630       1639       1648       1657       1666       1675
GTT ATA GAT TGA TCA TTC TTT CTC CTG ATA ATA AAG GTA TTG AAT ATC TGT TAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1684
AAA AAA AAA AAA AAA 3'
--- --- --- --- ---
```

FIG. 2

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                9         18        27        36        45        54
5'  GCC CGG CGA GGG CGC CGG TGC TTT GTT CTG TCT GAG GCC AGG AAG TTT GAC CGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   R   R   G   R   R   C   F   V   L   S   E   A   R   K   F   D   R

               63        72        81        90        99       108
    GCT GCC ATG CCG AAC CGT AAG GCC AGC CGG AAT GCT TAC TAT TTC TTC GTG CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   A   M   P   N   R   K   A   S   R   N   A   Y   Y   F   F   V   Q

              117       126       135       144       153       162
    GAG AAG ATC CCC GAA CTA CGG CGA CGA GGC CTG CCT GTG GCT CGC GTT GCT GAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     E   K   I   P   E   L   R   R   R   G   L   P   V   A   R   V   A   D

              171       180       189       198       207       216
    GCC ATC CCT TAC TGC TCC TCA GAC TGG GCG AAA CCT GTT TTC ACA CCA CTG AGG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   I   P   Y   C   S   S   D   W   A   K   P   V   F   T   P   L   R

              225       234       243       252       261       270
    AGG CCA GGC ATG CTT GTA CCA AAG CAG AAT GTT TCA CCT CCA GAT ATG TCA GCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   P   G   M   L   V   P   K   Q   N   V   S   P   P   D   M   S   A

              279       288       297       306       315       324
    TTG TCT TTA AAA GGT GAT CAA GCT CTC CTT GGA GGC ATT TTT TAT TTT TTG AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     L   S   L   K   G   D   Q   A   L   L   G   G   I   F   Y   F   L   N

              333       342       351       360       369       378
    ATT TTT AGC CAT GGC GAG CTA CCT CCT CAT TGT GAA CAG CGC TTC CTC CCT TGT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     I   F   S   H   G   E   L   P   P   H   C   E   Q   R   F   L   P   C

              387       396       405       414       423       432
    GAA ATT GGC TGT GTT AAG TAT TCT CTC CAA GAA GGT ATT ATG GCA GAT TTC CAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     E   I   G   C   V   K   Y   S   L   Q   E   G   I   M   A   D   F   H

              441       450       459       468       477       486
    AGT TTT ATA AAT CCT GGT GAA ATT CCA CGA GGA TTT CGA TTT CAT TGT CAG GCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     S   F   I   N   P   G   E   I   P   R   G   F   R   F   H   C   Q   A

              495       504       513       522       531       540
    GCA AGT GAT TCT AGT CAC AAG ATT CCT ATT TCA AAT TTT GAA CGT GGG CAT AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     A   S   D   S   S   H   K   I   P   I   S   N   F   E   R   G   H   N

              549       558       567       576       585       594
    CAA GCA ACT GTG TTA CAA AAC CTT TAT AGA TTT ATT CAT CCC AAC CCA GGG AAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Q   A   T   V   L   Q   N   L   Y   R   F   I   H   P   N   P   G   N

              603       612       621       630       639       648
    TGG CCA CCT ATC TAC TGC AAG TCT GAT GAT AGA ACC AGA GTC AAC TGG TGT TTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     W   P   P   I   Y   C   K   S   D   D   R   T   R   V   N   W   C   L

              657       666       675       684       693       702
    AAG CAT ATG GCA AAG GCA TCA GAA ATC AGG CAA GAT CTA CAA CTT CTC ACT GTA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     K   H   M   A   K   A   S   E   I   R   Q   D   L   Q   L   L   T   V

              711       720       729       738       747       756
    GAG GAC CTT GTA GTG GGG ATC TAC CAA CAA AAA TTT CTC AAG GAG CCC TCT AAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     E   D   L   V   V   G   I   Y   Q   Q   K   F   L   K   E   P   S   K
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        765         774         783         792         801         810
ACT TGG ATT CGA AGC CTC CTA GAT GTG GCC ATG TGG GAT TAT TCT AGC AAC ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   W   I   R   S   L   L   D   V   A   M   W   D   Y   S   S   N   T

        819         828         837         846         855         864
AGG TGC AAG TGG CAT GAA GAA AAT GAT ATT CTC TTC TGT GCT TTA GCT GTT TGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 R   C   K   W   H   E   E   N   D   I   L   F   C   A   L   A   V   C

        873         882         891         900         909         918
AAG AAG ATT GCG TAC TGC ATC AGT AAT TCT CTG GCC ACT CTC TTT GGA ATC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   K   I   A   Y   C   I   S   N   S   L   A   T   L   F   G   I   Q

        927         936         945         954         963         972
CTC ACA GAG GCT CAT GTA CCA CTA CAA GAT TAT GAG GCC AGC AAT AGT GTG ACA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   T   E   A   H   V   P   L   Q   D   Y   E   A   S   N   S   V   T

        981         990         999        1008        1017        1026
CCC AAA ATG GTT GTA TTG GAT GCA GGG CGT TAC CAG AAG CTA AGG GTT GGG AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 P   K   M   V   V   L   D   A   G   R   Y   Q   K   L   R   V   G   S

       1035        1044        1053        1062        1071        1080
TCA GGA TTC TCT CAT TTC AAC TCT TCT AAT GAG GAA CAA AGA TCA AAC ACA CCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 S   G   F   S   H   F   N   S   S   N   E   E   Q   R   S   N   T   P

       1089        1098        1107        1116        1125        1134
ATT GGT GAC TAC CCA TCT AGG GCA AAA ATT TCT GGC CAA AAC AGC AGC GTT CGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 I   G   D   Y   P   S   R   A   K   I   S   G   Q   N   S   S   V   R

       1143        1152        1161        1170        1179        1188
GGA AGA GGA ATT ACC CGC TTA CTA GAG AGC ATT TCC AAT TCT TCC AGC AAT ATC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   R   G   I   T   R   L   L   E   S   I   S   N   S   S   S   N   I

       1197        1206        1215        1224        1233        1242
CAC AAA TTC TCC AAC TGT GAC ACT TCA CTC TCA CCT TAC ATG TCC CAA AAA GAT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 H   K   F   S   N   C   D   T   S   L   S   P   Y   M   S   Q   K   D

       1251        1260        1269        1278        1287        1296
GGA TAC AAA TCT TTC TCT TCC TTA TCT TAA TGA TGG TAC TCT TTT CAA TTT CTG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 G   Y   K   S   F   S   S   L   S   *   *

       1305        1314        1323        1332        1341        1350
AAA ACA GTA ACA GGC CCA ACT TCC TTC TTA CTA CAG TCA TAT TAA ACA GAT CAC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1359        1368        1377        1386        1395        1404
ATC AAT GAC AAA TGT CAC TAC TAT AAA AAC TAC TTA ATT TGT AAG GAA ATT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1413        1422        1431        1440        1449        1458
TCA TAG ATT TTA AAA AAT TGT GGT TGG AGA GCA TCT TGG CAT TTG TGC TTT TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1467        1476        1485        1494        1503        1512
TCT TGA GGG ATT GTT CTG CTT CCT GGC TGT ATG ATG GGT ATA TCA TTA AAG TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1521        1530        1539        1548        1557        1566
GGA GTC CTA TAT GAA CAA AAC TGA CAT TTT TAG AGT TGT ACT TTT GGG AAT GTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1575        1584        1593        1602        1611        1620
ATA GAT TGA TCA TTC TTT CTC CTG ATA ATA AAG GTA TTG AAT ATC TGT TAT GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---

       1629        1638
AGG TTA AAA AAA AAA AA 3'
--- --- --- --- --- --
```

FIG. 3

```
                9          18          27          36          45          54
5'  GCG CGG CAC GGG GCG AGC GTC TCC CCG CCG CAG AGC CCG CCG CGC GGG GGA GCT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
               63          72          81          90          99         108
    CGG CCC GCC GCA CCG CCT CCC GCG CCT CCG CCC CGC CGC CCG CTG CCG CGA CTG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
              117         126         135         144         153         162
    CCA AAG TTT CTC GGT CAC GTG CTG GCC CCC GGC GGC CCA AAG GAG AAG ATC CCC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
              171         180         189         198         207         216
    GAA CTA CGG CGA CGA GGC CTG CCT GTG GCT CGC GTT GCT GAT GCC ATC CCT TAC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
              225         234         243         252         261         270
    TGC TCC TCA GAC TGG GCG CTT CTG AGG GAG GAA GAA AAG GAG AAA TAC GCA GAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
              279         288         297         306         315         324
    ATG GCT CGA GAA TGG AGG GCC GCT CAG GGA AAG GAC CCT GGG CCC TCA GAG AAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     M   A   R   E   W   R   A   A   Q   G   K   D   P   G   P   S   E   K

              333         342         351         360         369         378
    CAG AAA CCT GTT TTC ACA CCA CTG AGG AGG CCA GGC ATG CTT GTA CCA AAG CAG
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Q   K   P   V   F   T   P   L   R   R   P   G   M   L   V   P   K   Q

              387         396         405         414         423         432
    AAT GTT TCA CCT CCA GAT ATG TCA GCT TTG TCT TTA AAA GCT CTC CTT GGA GGC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     N   V   S   P   P   D   M   S   A   L   S   L   K   A   L   L   G   G

              441         450         459         468         477         486
    ATT TTT TAT TTT TTG AAC ATT TTT AGC CAT GGC GAG CTA CCT CCT CAT TGT GAA
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     I   F   Y   F   L   N   I   F   S   H   G   E   L   P   P   H   C   E

              495         504         513         522         531         540
    CAG CGC TTC CTC CCT TGT GAA ATT GGC TGT GTT AAG TAT TCT CTC CAA GAA GGT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     Q   R   F   L   P   C   E   I   G   C   V   K   Y   S   L   Q   E   G

              549         558         567         576         585         594
    ATT ATG GCA GAT TTC CAC AGT TTT ATA AAT CCT GGT GAA ATT CCA CGA GGA TTT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     I   M   A   D   F   H   S   F   I   N   P   G   E   I   P   R   G   F

              603         612         621         630         639         648
    CGA TTT CAT TGT CAG GCT GCA AGT GAT TCT AGT CAC AAG ATT CCT ATT TCA AAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   F   H   C   Q   A   A   S   D   S   S   H   K   I   P   I   S   N

              657         666         675         684         693         702
    TTT GAA CGT GGG CAT AAC CAA GCA ACT GTG TTA CAA AAC CTT TAT AGA TTT ATT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     F   E   R   G   H   N   Q   A   T   V   L   Q   N   L   Y   R   F   I

              711         720         729         738         747         756
    CAT CCC AAC CCA GGG AAC TGG CCA CCT ATC TAC TGC AAG TCT GAT GAT AGA ACC
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     H   P   N   P   G   N   W   P   P   I   Y   C   K   S   D   D   R   T

              765         774         783         792         801         810
    AGA GTC AAC TGG TGT TTG AAG CAT ATG GCA AAG GCA TCA GAA ATC AGG CAA GAT
    --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
     R   V   N   W   C   L   K   H   M   A   K   A   S   E   I   R   Q   D
```

```
        819         828         837         846         855         864
CTA CAA CTT CTC ACT GTA GAG GAC CTT GTA GTG GGG ATC TAC CAA CAA AAA TTT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   Q   L   L   T   V   E   D   L   V   V   G   I   Y   Q   Q   K   F

        873         882         891         900         909         918
CTC AAG GAG CCC TCT AAG ACT TGG ATT CGA AGC CTC CTA GAT GTG GCC ATG TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 L   K   E   P   S   K   T   W   I   R   S   L   L   D   V   A   M   W

        927         936         945         954         963         972
GAT TAT TCT AGC AAC ACA AGG TGC AAG TGG CAT GAA GAA AAT GAT ATT CTC TTC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 D   Y   S   S   N   T   R   C   K   W   H   E   E   N   D   I   L   F

        981         990         999        1008        1017        1026
TGT GCT TTA GCT GTT TGC AAG AAG ATT GCG TAC TGC ATC AGT AAT TCT CTG GCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 C   A   L   A   V   C   K   K   I   A   Y   C   I   S   N   S   L   A

       1035        1044        1053        1062        1071        1080
ACT CTC TTT GGA ATC CAG CTC ACA GAG GCT CAT GTA CCA CTA CAA GAT TAT GAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 T   L   F   G   I   Q   L   T   E   A   H   V   P   L   Q   D   Y   E

       1089        1098        1107        1116        1125        1134
GCC AGC AAT AGT GTG ACA CCC AAA ATG GTT GTA TTG GAT GCA GGG CGT TAC CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 A   S   N   S   V   T   P   K   M   V   V   L   D   A   G   R   Y   Q

       1143        1152        1161        1170        1179        1188
AAG CTA AGG GTT GGG AGT TCA GGA TTC TCT CAT TTC AAC TCT TCT AAT GAG GAA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 K   L   R   V   G   S   S   G   F   S   H   F   N   S   S   N   E   E

       1197        1206        1215        1224        1233        1242
CAA AGA TCA AAC ACA CCC ATT GGT GAC TAC CCA TCT AGG GCA AAA ATT TCT GGC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   R   S   N   T   P   I   G   D   Y   P   S   R   A   K   I   S   G

       1251        1260        1269        1278        1287        1296
CAA AAC AGC AGC GTT CGG GGA AGA GGA ATT ACC CGC TTA CTA GAG AGC ATT TCC
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Q   N   S   S   V   R   G   R   G   I   T   R   L   L   E   S   I   S

       1305        1314        1323        1332        1341        1350
AAT TCT TCC AGC AAT ATC CAC AAA TTC TCC AAC TGT GAC ACT TCA CTC TCA CCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 N   S   S   S   N   I   H   K   F   S   N   C   D   T   S   L   S   P

       1359        1368        1377        1386        1395        1404
TAC ATG TCC CAA AAA GAT GGA TAC AAA TCT TTC TCT TCC TTA TCT TAA TGA TGG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
 Y   M   S   Q   K   D   G   Y   K   S   F   S   S   L   S   *   *

       1413        1422        1431        1440        1449        1458
TAC TCT TTT CAA TTT CTG AAA ACA GTA ACA GGC CCA ACT TCC TTC TTA CTA CAG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       1467        1476        1485        1494        1503        1512
TCA TAT TAA ACA GAT CAC ATC AAT GAC AAA TGT CAC TAC TAT AAA AAC TAC TTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       1521        1530        1539        1548        1557        1566
ATT TGT AAG GAA ATT GTT TCA TAG ATT TAA AAA AAT TGT GGT TGG AGA GCA TCT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
       1575        1584        1593        1602        1611        1620
TGG CAT TTG TGC TTT TTT TCT TGA GGG ATT GTT CTG CTT CCT GGC TGT ATG ATG
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
```

```
      1629        1638        1647        1656        1665        1674
GGT ATA TCA TTA AAG TTT GGA GTC CTA TAT GAA CAA AAC TGA CAT TTT TAG AGT
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1683        1692        1701        1710        1719        1728
TGT ACT TTT GGG AAT GTT ATA GAT TGA TCA TTC TTT CTC CTG ATA ATA AAG GTA
--- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- --- ---
      1737        1746        1755
TTG AAT ATC TGT TAA AAA AAA AAA AAA AAA AA 3'
--- --- --- --- --- --- --- --- --- --- --
```

FIG 4

GATCTTGCCTGATTTCTGATGCCTTTGCCATATGCTTCAAACACCAGTTGACTCTGGTTCTATCATCAGACTTGCA
GTAGATAGGTGGTCAGTTCCCTGGGTTGGGATGAATAAATCTATAAAGGTTTTGTAACACAGTTGCTTGGTTATGC
CCACGTTCAAAATTTGAAATAGGAATCTTGTGACTAGAATCACTTGCAGCCTGACAATGAAATCGAAATCCTCGTG
GAATTTCACCAGGATTTATAAAACTGTGGAAATCTGCCATAATACCTTCTTGGAGAGAATACTTAACACAGCCAAT
TTCACAAGGGAGGAAGCGCTGTTCACAATGAGGAGGTAGCTCGCCATGGCTAAAAATGTTCAAAAAATAAAAAATG
CCTCCAAGGAGAGCTTTTAAAGACAAAGCTGACATATCTGGAGGTGAAACATTCTGCTTTGGTACAAGCATGCCTG
GCCTCCTCAG

FIG. 5

```
          1               15 16              30 31              45 46              60 61              75 76              90
1 PTAN-1 MPNRKASRNAYYFFV QEKIPELRRRGLPVA RVADAIPYCSSDWAL LREEEKEKYAEMARE WRAAQGKDPGPSEKQ KPVFTPLRRPGMLVP      90
2 PTAN-2 MPNRKASRNAYYFFV QEKIPELRRRGLPVA RVADAIPYCSSDWA- ---------------- ---------------- KPVFTPLRRPGMLVP      59
3 PTAN-3                                                                   MARE WRAAQGKDPGPSEKQ KPVFTPLRRPGMLVP      34

          91             105 106             120 121             135 136             150 151             165 166             180
1 PTAN-1 KQNVSPPDMSALSLK GDQALLGGIFYFLNI FSHGELPPHCEQRFL PCEIGCVKYSLQEGI MADFHSFINPGEIPR GFRFHCQAASDSSHK     180
2 PTAN-2 KQNVSPPDMSALSLK GDQALLGGIFYFLNI FSHGELPPHCEQRFL PCEIGCVKYSLQEGI MADFHSFINPGEIPR GFRFHCQAASDSSHK     149
3 PTAN-3 KQNVSPPDMSALSLK ---ALLGGIFYFLNI FSHGELPPHCEQRFL PCEIGCVKYSLQEGI MADFHSFINPGEIPR GFRFHCQAASDSSHK     121

          181            195 196            210 211            225 226            240 241            255 256            270
1 PTAN-1 IPISNFERGHNQATV LQNLYRFIHPNPGNW PPIYCKSDDRTRVNW CLKHMAKASEIRQDL QLLTVEDLVVGIYQQ KFLKEPSKTWIRSLL     270
2 PTAN-2 IPISNFERGHNQATV LQNLYRFIHPNPGNW PPIYCKSDDRTRVNW CLKHMAKASEIRQDL QLLTVEDLVVGIYQQ KFLKEPSKTWIRSLL     239
3 PTAN-3 IPISNFERGHNQATV LQNLYRFIHPNPGNW PPIYCKSDDRTRVNW CLKHMAKASEIRQDL QLLTVEDLVVGIYQQ KFLKEPSKTWIRSLL     211

          271            285 286            300 301            315 316            330 331            345 346            360
1 PTAN-1 DVAMWDYSSNTRCKW HEENDILFCALAVCK KIAYCISNSLATLFG IQLTEAHVPLQDYEA SNSVTPKMVVLDAGR YQKLRVGSSGFSHFN     360
2 PTAN-2 DVAMWDYSSNTRCKW HEENDILFCALAVCK KIAYCISNSLATLFG IQLTEAHVPLQDYEA SNSVTPKMVVLDAGR YQKLRVGSSGFSHFN     329
3 PTAN-3 DVAMWDYSSNTRCKW HEENDILFCALAVCK KIAYCISNSLATLFG IQLTEAHVPLQDYEA SNSVTPKMVVLDAGR YQKLRVGSSGFSHFN     301

          361            375 376            390 391            405 406            420 421            435 436            450
1 PTAN-1 SSNEEQRSNTPIGDY PSRAKISGQNSSVRG RGITRLLESISNSSS NIHKFSNCDTSLSPY MSQKDGYKSFSSLS     434
2 PTAN-2 SSNEEQRSNTPIGDY PSRAKISGQNSSVRG RGITRLLESISNSSS NIHKFSNCDTSLSPY MSQKDGYKSFSSLS     403
3 PTAN-3 SSNEEQRSNTPIGDY PSRAKISGQNSSVRG RGITRLLESISNSSS NIHKFSNCDTSLSPY MSQKDGYKSFSSLS     375
```

FIG. 6

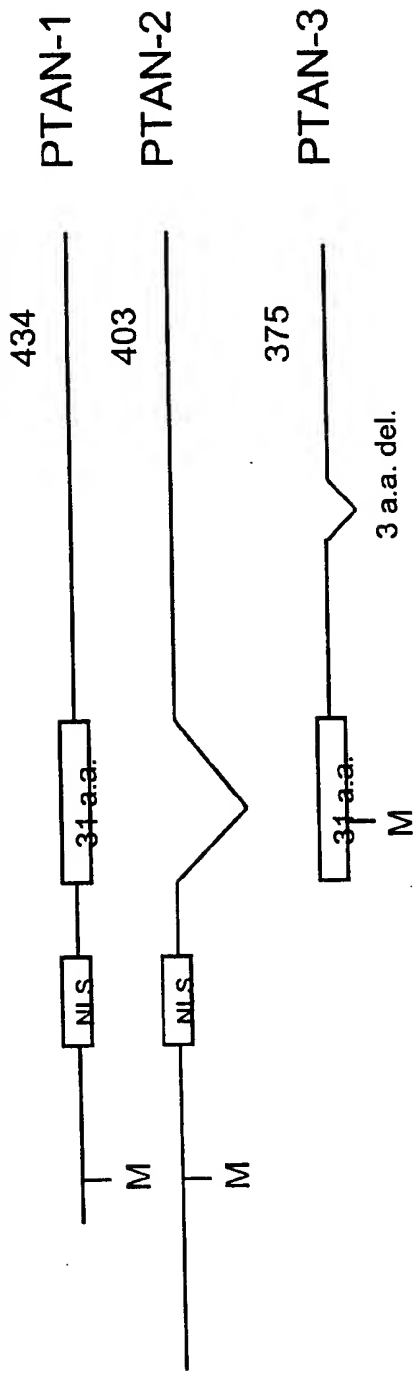
PTAN-1
PTAN-2
PTAN-3
434
403
375
31 a.a.
NLS
M
3 a.a. del.
NLS= nuclear localization signal

FIG. 7

```
          1              15 16             30 31             45 46             60 61             75 76             90
1 PTAN-1  ---------------- ---------------- ----------GGCCA GGA--AGTTTGACCG CGCTGC----CATGC CGAACCGTAAGGCCA      44
2 PTAN-2  -----GCCCGGCGAG GGCGCCGGTGCTTTG TTCTGTCTGAGGCCA GGA--AGTTTGACCG CGCTGC----CATGC CGAACCGTAAGGCCA      79
3 PTAN-3  GCGCGGCACGGGGCG AGCGTCTCCCCGCCG CAGAGCCCGCCGCGC GGGGGAGCTCGGCC- CGCCGCACCGCCTCC CGCGCC-TCCGCCCC      88

          91            105 106           120 121           135 136           150 151           165 166           180
1 PTAN-1  GCCG------------ GAATGCTTACTATTT CTT-----CGTGC-- ---------------- -AGGAGAAGATCCCC GAACTACGGCGACGA     100
2 PTAN-2  GCCG------------ GAATGCTTACTATTT CTT-----CGTGC-- ---------------- -AGGAGAAGATCCCC GAACTACGGCGACGA     135
3 PTAN-3  GCCGCCCGCTGCCGC GACTGCCAAAG-TTT CTCGGTCACGTGCTG GCCCCCGGCGGCCCA AAGGAGAAGATCCCC GAACTACGGCGACGA     177

          181           195 196           210 211           225 226           240 241           255 256           270
1 PTAN-1  GGCCTGCCTGTGGCT CGCGTTGCTGATGCC ATCCCTTACTGCTCC TCAGACTGGGCGCTT CTGAGGGAGGAAGAA AAGGAGAAATACGCA     190
2 PTAN-2  GGCCTGCCTGTGGCT CGCGTTGCTGATGCC ATCCCTTACTGCTCC TCAGACTGGGCG--- ---------------- ----------------     192
3 PTAN-3  GGCCTGCCTGTGGCT CGCGTTGCTGATGCC ATCCCTTACTGCTCC TCAGACTGGGCGCTT CTGAGGGAGGAAGAA AAGGAGAAATACGCA     267

          271           285 286           300 301           315 316           330 331           345 346           360
1 PTAN-1  GAAATGGCTCGAGAA TGGAGGGCCGCTCAG GGAAAGGACCCTGGG CCCTCAGAGAAGCAG AAACCTGTTTTCACA CCACTGAGGAGGCCA     280
2 PTAN-2  ---------------- ---------------- ---------------- ---------------- AAACCTGTTTTCACA CCACTGAGGAGGCCA     222
3 PTAN-3  GAAATGGCTCGAGAA TGGAGGGCCGCTCAG GGAAAGGACCCTGGG CCCTCAGAGAAGCAG AAACCTGTTTTCACA CCACTGAGGAGGCCA     357
```

```
          361        375 376        390 391        405 406        420 421        435 436        450
1 PTAN-1 GGCATGCTTGTACCA AAGCAGAATGTTTCA CCTCCAGATATGTCA GCTTTGTCTTTAAAA GGTGATCAAGCTCTC CTTGGAGGCATTTTT   370
2 PTAN-2 GGCATGCTTGTACCA AAGCAGAATGTTTCA CCTCCAGATATGTCA GCTTTGTCTTTAAAA GGTGATCAAGCTCTC CTTGGAGGCATTTTT   312
3 PTAN-3 GGCATGCTTGTACCA AAGCAGAATGTTTCA CCTCCAGATATGTCA GCTTTGTCTTTAAAA G---------CTCTC CTTGGAGGCATTTTT   438

          451        465 466        480 481        495 496        510 511        525 526        540
1 PTAN-1 TATTTTTTGAACATT TTTAGCCATGGCGAG CTACCTCCTCATTGT GAACAGCGCTTCCTC CCTTGTGAAATTGGC TGTGTTAAGTATTCT   460
2 PTAN-2 TATTTTTTGAACATT TTTAGCCATGGCGAG CTACCTCCTCATTGT GAACAGCGCTTCCTC CCTTGTGAAATTGGC TGTGTTAAGTATTCT   402
3 PTAN-3 TATTTTTTGAACATT TTTAGCCATGGCGAG CTACCTCCTCATTGT GAACAGCGCTTCCTC CCTTGTGAAATTGGC TGTGTTAAGTATTCT   528

          541        555 556        570 571        585 586        600 601        615 616        630
1 PTAN-1 CTCCAAGAAGGTATT ATGGCAGATTTCCAC AGTTTTATAAATCCT GGTGAAATTCCACGA GGATTTCGATTTCAT TGTCAGGCTGCAAGT   550
2 PTAN-2 CTCCAAGAAGGTATT ATGGCAGATTTCCAC AGTTTTATAAATCCT GGTGAAATTCCACGA GGATTTCGATTTCAT TGTCAGGCTGCAAGT   492
3 PTAN-3 CTCCAAGAAGGTATT ATGGCAGATTTCCAC AGTTTTATAAATCCT GGTGAAATTCCACGA GGATTTCGATTTCAT TGTCAGGCTGCAAGT   618

          631        645 646        660 661        675 676        690 691        705 706        720
1 PTAN-1 GATTCTAGTCACAAG ATTCCTATTTCAAAT TTTGAACGTGGGCAT AACCAAGCAACTGTG TTACAAAACCTTTAT AGATTTATTCATCCC   640
2 PTAN-2 GATTCTAGTCACAAG ATTCCTATTTCAAAT TTTGAACGTGGGCAT AACCAAGCAACTGTG TTACAAAACCTTTAT AGATTTATTCATCCC   582
3 PTAN-3 GATTCTAGTCACAAG ATTCCTATTTCAAAT TTTGAACGTGGGCAT AACCAAGCAACTGTG TTACAAAACCTTTAT AGATTTATTCATCCC   708

          721        735 736        750 751        765 766        780 781        795 796        810
1 PTAN-1 AACCCAGGGAACTGG CCACCTATCTACTGC AAGTCTGATGATAGA ACCAGAGTCAACTGG TGTTTGAAGCATATG GCAAAGGCATCAGAA   730
2 PTAN-2 AACCCAGGGAACTGG CCACCTATCTACTGC AAGTCTGATGATAGA ACCAGAGTCAACTGG TGTTTGAAGCATATG GCAAAGGCATCAGAA   672
3 PTAN-3 AACCCAGGGAACTGG CCACCTATCTACTGC AAGTCTGATGATAGA ACCAGAGTCAACTGG TGTTTGAAGCATATG GCAAAGGCATCAGAA   798

          811        825 826        840 841        855 856        870 871        885 886        900
1 PTAN-1 ATCAGGCAAGATCTA CAACTTCTCACTGTA GAGGACCTTGTAGTG GGGATCTACCAACAA AAATTTCTCAAGGAG CCCTCTAAGACTTGG   820
2 PTAN-2 ATCAGGCAAGATCTA CAACTTCTCACTGTA GAGGACCTTGTAGTG GGGATCTACCAACAA AAATTTCTCAAGGAG CCCTCTAAGACTTGG   762
3 PTAN-3 ATCAGGCAAGATCTA CAACTTCTCACTGTA GAGGACCTTGTAGTG GGGATCTACCAACAA AAATTTCTCAAGGAG CCCTCTAAGACTTGG   888
```

```
                 901       915 916       930 931       945 946       960 961       975 976       990
1 PTAN-1 ATTCGAAGCCTCCTA GATGTGGCCATGTGG GATTATTCTAGCAAC ACAAGGTGCAAGTGG CATGAAGAAAATGAT ATTCTCTTCTGTGCT  910
2 PTAN-2 ATTCGAAGCCTCCTA GATGTGGCCATGTGG GATTATTCTAGCAAC ACAAGGTGCAAGTGG CATGAAGAAAATGAT ATTCTCTTCTGTGCT  852
3 PTAN-3 ATTCGAAGCCTCCTA GATGTGGCCATGTGG GATTATTCTAGCAAC ACAAGGTGCAAGTGG CATGAAGAAAATGAT ATTCTCTTCTGTGCT  978

                  991      1005 1006      1020 1021      1035 1036      1050 1051      1065 1066      1080
1 PTAN-1 TTAGCTGTTTGCAAG AAGATTGCGTACTGC ATCAGTAATTCTCTG GCCACTCTCTTTGGA ATCCAGCTCACAGAG GCTCATGTACCACTA 1000
2 PTAN-2 TTAGCTGTTTGCAAG AAGATTGCGTACTGC ATCAGTAATTCTCTG GCCACTCTCTTTGGA ATCCAGCTCACAGAG GCTCATGTACCACTA  942
3 PTAN-3 TTAGCTGTTTGCAAG AAGATTGCGTACTGC ATCAGTAATTCTCTG GCCACTCTCTTTGGA ATCCAGCTCACAGAG GCTCATGTACCACTA 1068

                 1081      1095 1096      1110 1111      1125 1126      1140 1141      1155 1156      1170
1 PTAN-1 CAAGATTATGAGGCC AGCAATAGTGTGACA CCCAAAATGGTTGTA TTGGATGCAGGGCGT TACCAGAAGCTAAGG GTTGGGAGTTCAGGA 1090
2 PTAN-2 CAAGATTATGAGGCC AGCAATAGTGTGACA CCCAAAATGGTTGTA TTGGATGCAGGGCGT TACCAGAAGCTAAGG GTTGGGAGTTCAGGA 1032
3 PTAN-3 CAAGATTATGAGGCC AGCAATAGTGTGACA CCCAAAATGGTTGTA TTGGATGCAGGGCGT TACCAGAAGCTAAGG GTTGGGAGTTCAGGA 1158

                 1171      1185 1186      1200 1201      1215 1216      1230 1231      1245 1246      1260
1 PTAN-1 TTCTCTCATTTCAAC TCTTCTAATGAGGAA CAAAGATCAAACACA CCCATTGGTGACTAC CCATCTAGGGCAAAA ATTTCTGGCCAAAAC 1180
2 PTAN-2 TTCTCTCATTTCAAC TCTTCTAATGAGGAA CAAAGATCAAACACA CCCATTGGTGACTAC CCATCTAGGGCAAAA ATTTCTGGCCAAAAC 1122
3 PTAN-3 TTCTCTCATTTCAAC TCTTCTAATGAGGAA CAAAGATCAAACACA CCCATTGGTGACTAC CCATCTAGGGCAAAA ATTTCTGGCCAAAAC 1248

                 1261      1275 1276      1290 1291      1305 1306      1320 1321      1335 1336      1350
1 PTAN-1 AGCAGCGTTCGGGGA AGAGGAATTACCCGC TTACTAGAGAGCATT TCCAATTCTTCCAGC AATATCCACAAATTC TCCAACTGTGACACT 1270
2 PTAN-2 AGCAGCGTTCGGGGA AGAGGAATTACCCGC TTACTAGAGAGCATT TCCAATTCTTCCAGC AATATCCACAAATTC TCCAACTGTGACACT 1212
3 PTAN-3 AGCAGCGTTCGGGGA AGAGGAATTACCCGC TTACTAGAGAGCATT TCCAATTCTTCCAGC AATATCCACAAATTC TCCAACTGTGACACT 1338
```

```
          1351       1365 1366       1380 1381       1395 1396       1410 1411       1425 1426       1440
1 PTAN-1 TCACTCTCACCTTAC ATGTCCCAAAAAGAT GGATACAAATCTTTC TCTTCCTTATCTTAA TGATGGTACTCTTTT CAATTTCTGAAAACA   1360
2 PTAN-2 TCACTCTCACCTTAC ATGTCCCAAAAAGAT GGATACAAATCTTTC TCTTCCTTATCTTAA TGATGGTACTCTTTT CAATTTCTGAAAACA   1302
3 PTAN-3 TCACTCTCACCTTAC ATGTCCCAAAAAGAT GGATACAAATCTTTC TCTTCCTTATCTTAA TGATGGTACTCTTTT CAATTTCTGAAAACA   1428

          1441       1455 1456       1470 1471       1485 1486       1500 1501       1515 1516       1530
1 PTAN-1 GTAACAGGCCCAACT TCCTTCTTACTACAG TCATATTAAACAGAT CACATCAATGACAAA TGTCACTACTATAAA AACTACTTAATTTGT   1450
2 PTAN-2 GTAACAGGCCCAACT TCCTTCTTACTACAG TCATATTAAACAGAT CACATCAATGACAAA TGTCACTACTATAAA AACTACTTAATTTGT   1392
3 PTAN-3 GTAACAGGCCCAACT TCCTTCTTACTACAG TCATATTAAACAGAT CACATCAATGACAAA TGTCACTACTATAAA AACTACTTAATTTGT   1518

          1531       1545 1546       1560 1561       1575 1576       1590 1591       1605 1606       1620
1 PTAN-1 AAGGAAATTGTTTCA TAGATTTAAAAAAAT TGTGGTTGGAGAGCA TCTTGGCATTTGTGC TTTTTTTCTTGAGGG ATTGTTCTGCTTCCT   1540
2 PTAN-2 AAGGAAATTGTTTCA TAGATTTTAAAAAAT TGTGGTTGGAGAGCA TCTTGGCATTTGTGC TTTTTTTCTTGAGGG ATTGTTCTGCTTCCT   1482
3 PTAN-3 AAGGAAATTGTTTCA TAGATTTAAAAAAAT TGTGGTTGGAGAGCA TCTTGGCATTTGTGC TTTTTTTCTTGAGGG ATTGTTCTGCTTCCT   1608

          1621       1635 1636       1650 1651       1665 1666       1680 1681       1695 1696       1710
1 PTAN-1 GGCTGTATGATGGGT ATATCATTAAAGTTT GGAGTCCTATATGAA CAAAACTGACATTTT TAGAGTTGTACTTTT GGGAATGTTATAGAT   1630
2 PTAN-2 GGCTGTATGATGGGT ATATCATTAAAGTTT GGAGTCCTATATGAA CAAAACTGACATTTT TAGAGTTGTACTTTT GGGAATGTTATAGAT   1572
3 PTAN-3 GGCTGTATGATGGGT ATATCATTAAAGTTT GGAGTCCTATATGAA CAAAACTGACATTTT TAGAGTTGTACTTTT GGGAATGTTATAGAT   1698

          1711       1725 1726       1740 1741       1755 1756       1770 1771       1785 1786       1800
1 PTAN-1 TGATCATTCTTTCTC CTGATAATAAAGGTA TTGAATATCTGTTA- -AAA----AAAAAAA AAAAAA--   1690
2 PTAN-2 TGATCATTCTTTCTC CTGATAATAAAGGTA TTGAATATCTGTTAT GAAAGGTTAAAAAAA AAAAAAAA   1640
3 PTAN-3 TGATCATTCTTTCTC CTGATAATAAAGGTA TTGAATATCTGTTA- -AAA----AAAAAAA AAAAAAAA   1760
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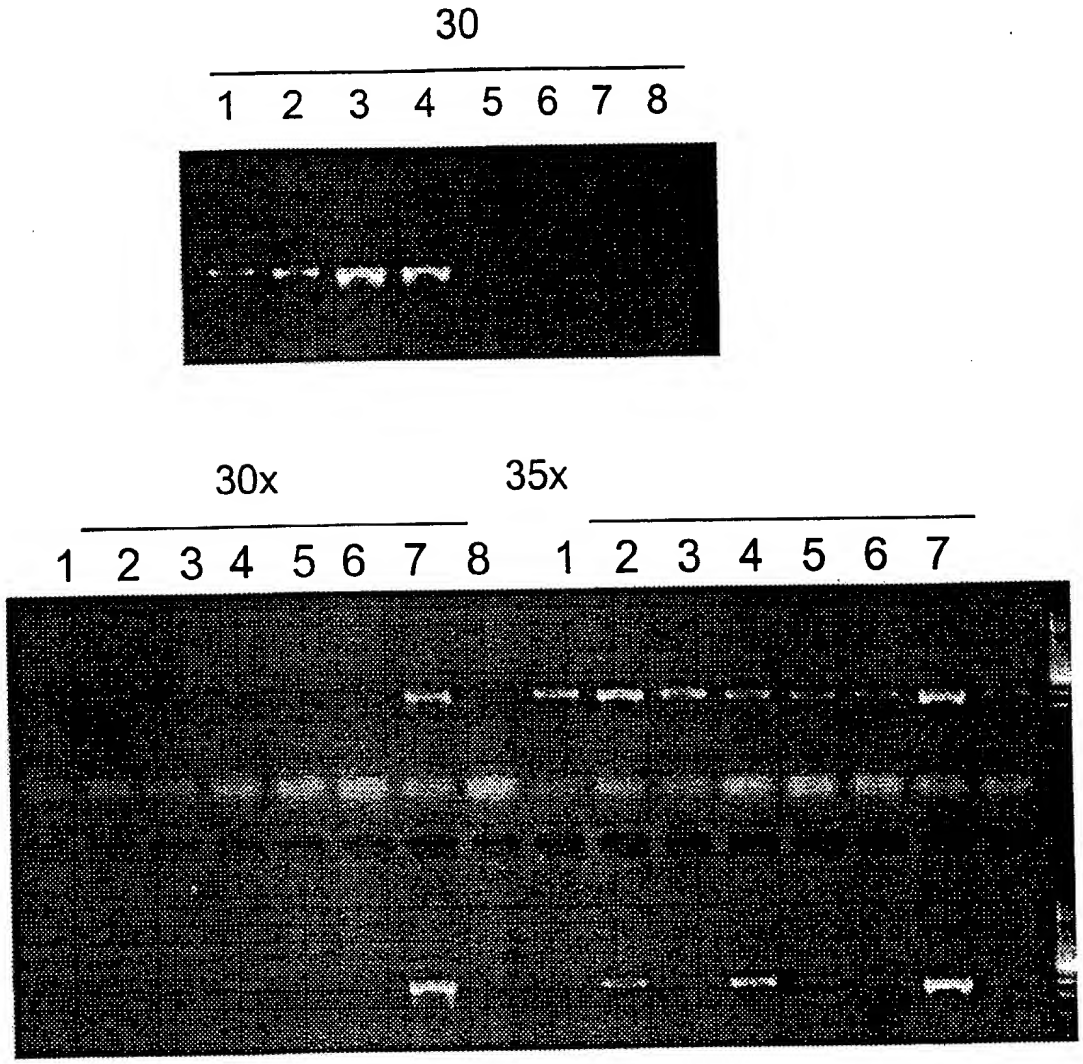
FIG. 8



Panels:

A

1. Brain
2. Prostate
3. LAPC-4 AD
4. LAPC-4 AI
5. LAPC-9 AD
6. HeLa
7. Murine cDNA
8. Neg. control

B

1. Brain
2. Heart
3. Kidney
4. Liver
5. Lung
6. Pancreas
7. Placenta
8. Skeletal Muscle

C

1. Colon
2. Ovary
3. Leukocytes
4. Prostate
5. Small Intestine
6. Spleen
7. Testis
8. Thymus

FIG. 9

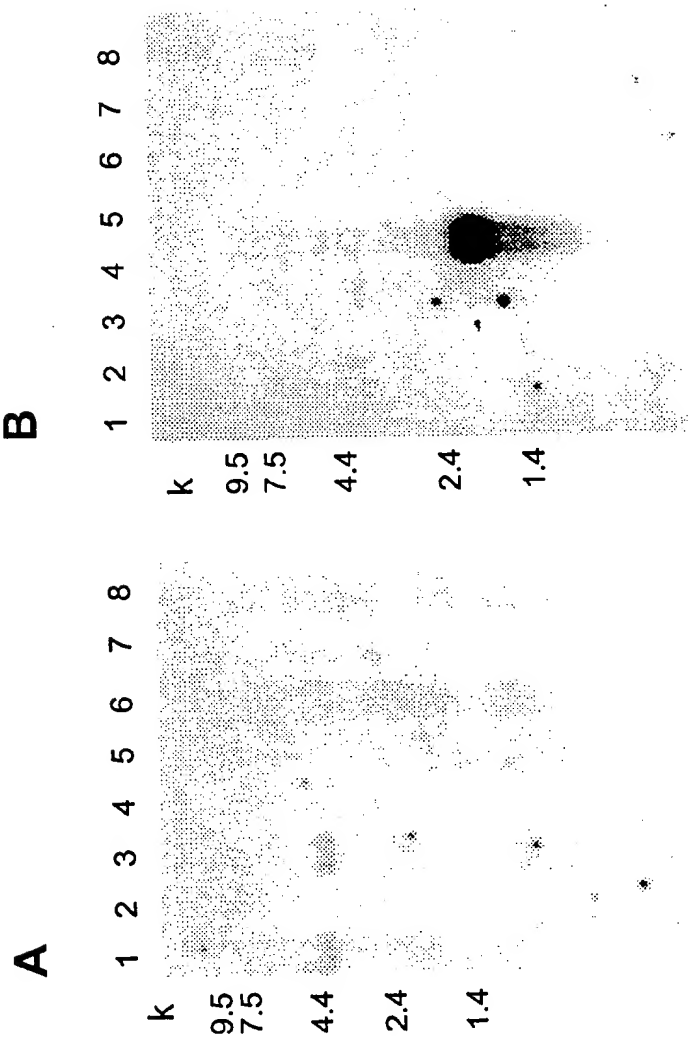
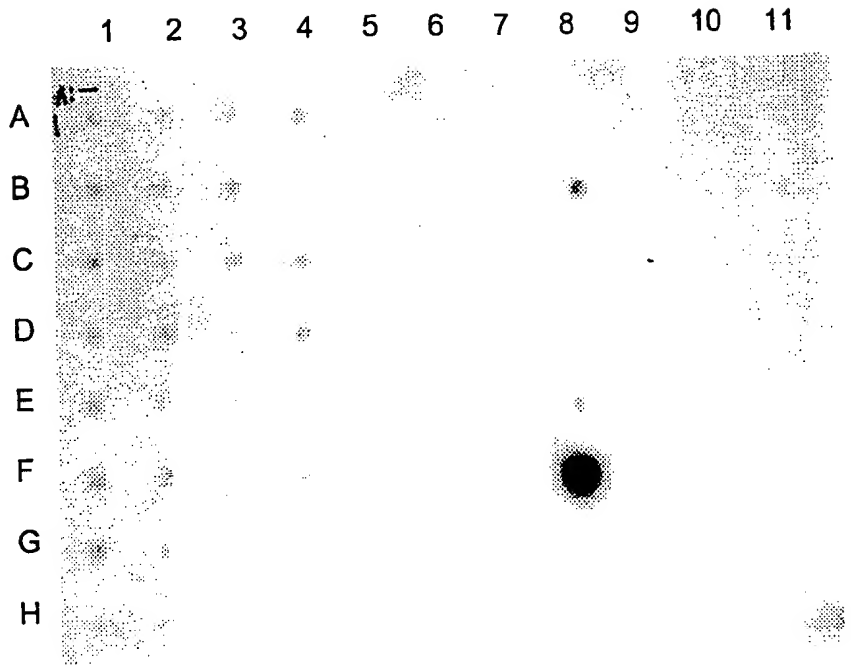

A
1 2 3 4 5 6 7 8
k
9.5
7.5
4.4
2.4
1.4
B
1 2 3 4 5 6 7 8
k
9.5
7.5
4.4
2.4
1.4
A
1. Heart
2. Brain
3. Placenta
4. Lung
5. Liver
6. Skeletal Muscle
7. Kidney
8. Pancreas
B
1. Spleen
2. Thymus
3. Prostate
4. Testis
5. Ovary
6. Small Intestine
7. Colon
8. Leukocytes

FIG. 10



A1 whole brain	B1 cerebral cortex	C1 frontal lobe	D1 parietal lobe
A2 cerebellum, left	B2 cerebellum, right	C2 corpus callosum	D2 amygdala
A3 substantia nigra	B3 accumbens nucleus	C3 thalamus	D3 pituitary gland
A4 heart	B4 aorta	C4 atrium, left	D4 atrium, right
A5 esophagus	B5 stomach	C5 duodenum	D5 jejunum
A6 colon, transverse	B6 colon, descending	C6 rectum	D6 -
A7 kidney	B7 skeletal muscle	C7 spleen	D7 thymus
A8 lung	B8 placenta	C8 bladder	D8 uterus
A9 liver	B9 pancreas	C9 adrenal gland	D9 thyroid gland
A10 HL60, leukemia	B10 HeLa, S3	C10 K562, leukemia	D10 MOLT-4, leukemia
A11fetal brain	B11fetal heart	C11 fetal kidney	D11 fetal liver

E1 occipital lobe	F1 temporal lobe	G1 paracentral gyrus	H1 pons
E2 caudate nucleus	F2 hippocampus	G2 medulla oblongata	H2 putamen
E3 spinal cord	F3 -	G3 -	H3 -
E4 ventricle, left	F4 ventricle, right	G4 interventricular septum	H4 apex of the heart
E5 ileum	F5 ilocecum	G5 appendix	H5 colon, ascending
E6 -	F6 -	G6 -	H6 -
E7 leukocytes	F7 lymph node	G7 bone marrow	H7 trachea
E8 prostate	**F8 testis**	G8 ovary	H8 -
E9 salivary gland	F9 mammary gland	G9 -	H9 -
E10 RAJI, lymphoma	F10 DAUDI, lymphoma	G10 SW480, colon cancer	H10 A549, lung cancer
E11 fetal spleen	F11 fetal thymus	G11 fetal lung	H11 -

FIG. 11

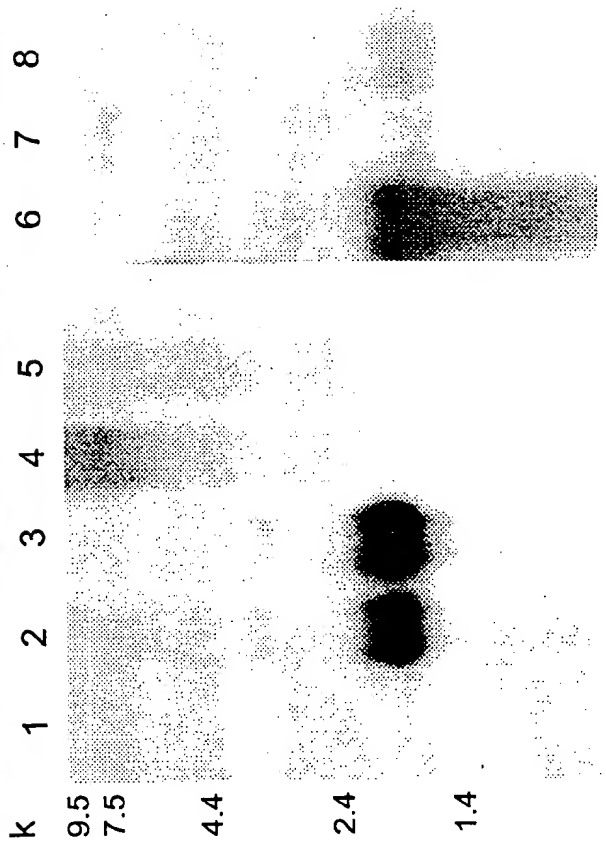

prostate
breast
1 2 3 4 5
6 7 8
k
9.5
7.5
4.4
2.4
1.4
1. Prostate
2. LAPC-4 AD
3. LAPC-4 AI
4. LAPC-9 AD
5. LAPC-9 AI
6. BT-20
7. CAMA-1
8. DU4475

FIG. 12

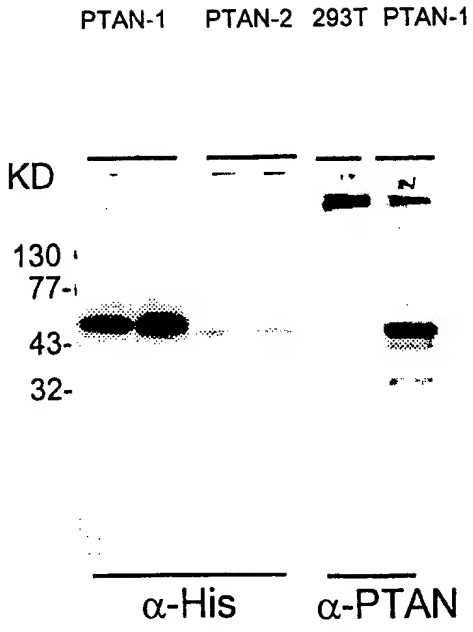
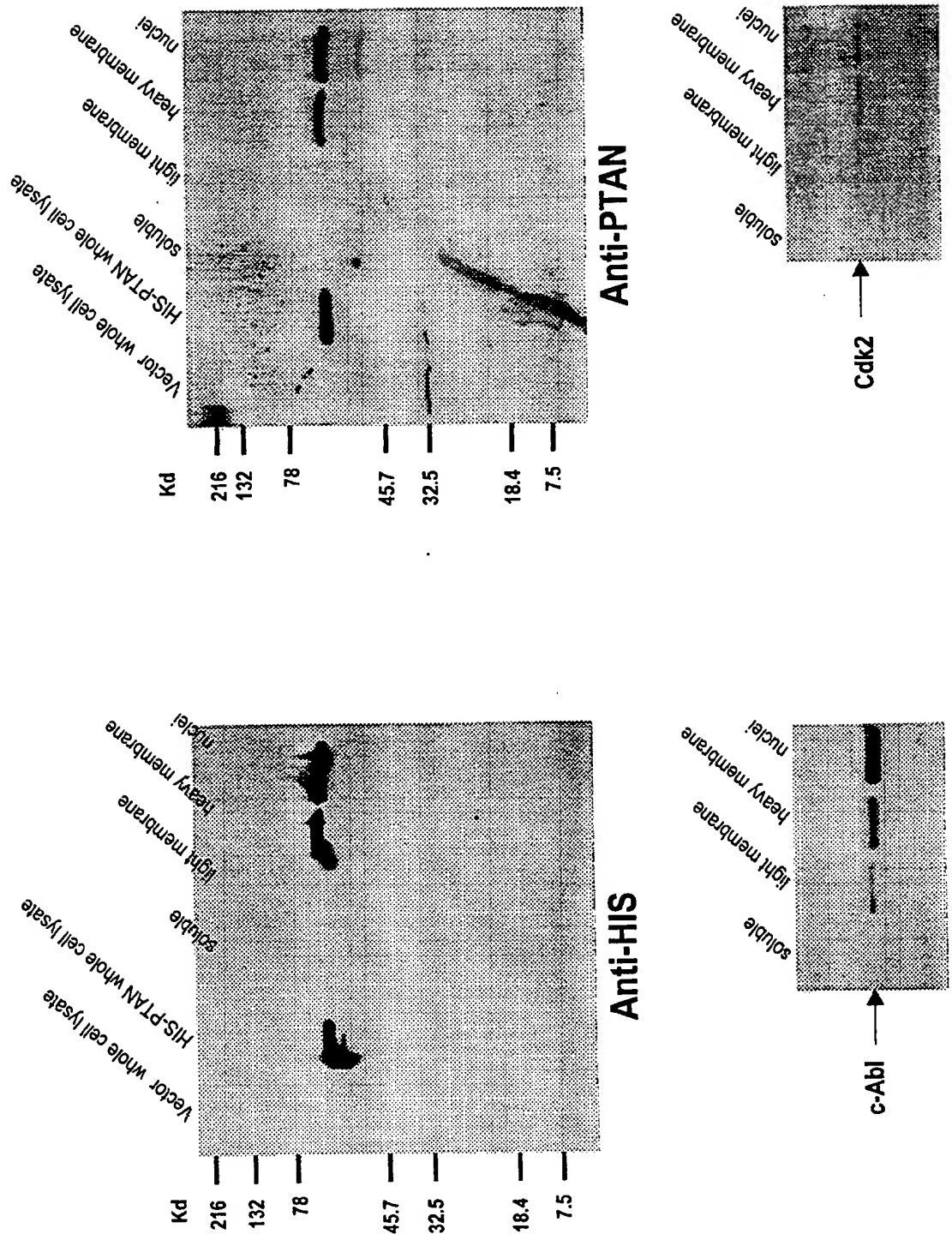


FIG. 13


Anti-PTAN
Vector whole cell lysate
HIS-PTAN whole cell lysate
soluble
light membrane
heavy membrane
nuclei
Kd
216
132
78
45.7
32.5
18.4
7.5
Cdk2
Anti-HIS
c-Abl

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/US 99/22985

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C12N5/10 C07K14/47 C07K16/18 G01N33/574
C12Q1/68 A61K38/17 A61K39/395 A01K67/027 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12N C07K A61K A01K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	DATABASE EMBL SEQUENCES 'Online! Accession No. AF012390, 7 January 1998 (1998-01-07) DE SMET C.: "H. testis H. sapiens cDNA clone TDP3.12b" XP002135705 complementary to cDNA fragment encoding amino acids 105-208 of seq. 4.	2,4,6-8, 11, 14-46, 48,49
A	-& DE SMETC. ET AL.: "Identification of human testis-specific transcripts and and analysis of their expression in tumor cells" BIOCHEM. BIOPHYS. RES. COMM., vol. 241, 1997, pages 653-567, XP002135703 the whole document	1-49

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 April 2000	08/05/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31–70) 340–2040, Tx. 31 651 epo nl, Fax: (+31–70) 340–3016	Galli, I

2

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	WO 98 16628 A (UNIV CALIFORNIA) 23 April 1998 (1998-04-23) abstract	1-49
A	-& KLEIN K.A. ET AL.: "Progression of metastatic human prostate cancer to androgen independence in immunodeficient SCID mice" NATURE MEDICINE, vol. 3, no. 4, April 1997 (1997-04), pages 402-408, XP002135704 cited in the application the whole document	1-49

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 99/22985

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 40-46 are directed to a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int tional Application No
PCT/US 99/22985

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9816628 A	23-04-1998	AU 4818997 A	11-05-1998
		EP 0953039 A	03-11-1999